STUDY OF SERUM FERRITIN LEVELS IN BLOOD DONORS

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Submitted By

DR. ARSHPREET KAUR BATRA

DEPARTMENT OF IMMUNOHEMATOLOGY AND BLOOD TRANSFUSION
DAYANAND MEDICAL COLLEGE AND HOSPITAL
LUDHIANA, PUNJAB

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_____

Dr. Arshpreet Kaur Batra
Department of IHBT,
Dayanand Medical College & Hospital,
Ludhiana, Punjab

Date:

Place:

CERTIFICATE OF SUPERVISORS

This is to certify that the work incorporated in this thesis entitled "**STUDY OF SERUM FERRITIN LEVELS IN BLOOD DONORS"** has been carried out by **Dr. Arshpreet Kaur Batra** under our direct supervision. The data incorporated in this thesis is original and genuine and has been carried out by the candidate herself.

Signature of Supervisor

Dr. Amarjit Kaur
Professor & Head
Department of IHBT
DMC&H, Ludhiana

Signature of Co-Supervisors

Dr. Navjot Bajwa
Professor & Head
Department of Biochemistry
DMC&H, Ludhiana

Dr. Rajesh Kumar
Professor
Department of IHBT
DMC&H, Ludhiana

Date:

ENDORSEMENT BY THE HEAD OF THE INSTITUTE

This is to certify that the thesis entitled "**STUDY OF SERUM FERRITIN LEVELS IN BLOOD DONORS"** is a bonafide research work done by **Dr. Arshpreet Kaur Batra** under the supervision of **Dr Amarjit Kaur**, Professor & Head, Department of IHBT, **Dr. Navjot Bajwa**, Professor & Head, Department of Biochemistry and **Dr. Rajesh Kumar**, Professor, Department of IHBT in this institution.

Principal

Dr. Sandeep Puri
Principal
DMC&H, Ludhiana

Date:

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Signature

Dr Arshpreet Kaur Batra
Department of IHBT,
Dayanand Medical College & Hospital,
Ludhiana, Punjab - 141001

Date:

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Dedicated
to
Arjas

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'A dream which became a reality'

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Dr. Arshpreet Kaur Batra

ABBREVIATIONS

VNRD	Voluntary Non Remunerated Blood Donation
WHO	World Health Organisation
EDQM	European Directorate for the Quality of Medicines & HealthCare
EBA	European Blood Alliance (EBA),
NGO	Non Governmental Organizations
ISBT	International Society of Blood Transfusion
FIODS	International Federation of Blood Donor Organizations
NACO	National AIDS Control Organization
NBTC	National Blood Tranfusion Council
Hb	Hemoglobin
IDA	Iron deficiency Anemia
ID	Iron Deficiency
TIBC	Total Iron Binding Capacity
BTS	Blood Transfusion Services
HIV	Human Immunodeficiency Virus
TTI	Transfusion Transmittible Infection
IRP 1	Iron Regulatory Protein 1
NCOA 4	Nuclear Receptor Coactivator 4
IDE	Iron Deficient Erythropoiesis
TBI	Total Body iron
RBC	Red Blood Cell
ECLIA	Electrochemiluminescent immunoassay

ABSTRACT

Background: Regular blood donations can lead to reduced iron stores in the body. Measurement of only hemoglobin levels is a poor indicator of reduced iron stores and Iron deficient erythropoiesis. The measurement of serum ferritin levels in regular voluntary donors helps to screen for subclinical iron deficiency. Early detection and timely interventions taken in case of subclinical iron deficiency can help to prevents symptomatic iron depletion and also helps in preventing the donor loss to the blood bank because of iron deficiency anemia.

Materials& methods: A prospective analysis of hemoglobin and serum ferritin levels in 200 donors coming to Department of Immunohematology and Blood Transfusion for blood donation was done. This included the measurement of the same parameters in a control group of 50 healthy donors donating for the first time. The donors included both sexes. A pattern between the interdonation interval, gender and age groups of the donors with the change in serum ferritin levels and hemoglobin was studied to assess the prevalence of subclinical iron deficiency.

Results: Hemoglobin and serum ferritin levels of a total of 200 donors(both males and females) were analysed in this study. With decreasing interdonation intervals, a significant decrement of serum ferritin levels was obtained($p<0.05$).It was seen that 0.5 % and 9 % of the total female and male donors respectively had serum ferritin levels <12 ng/ml. A significant correlation between the interdonation interval and the subsequent donations made by the male donors was seen in a follow up analysis.

Conclusion: An inverse relationship was found between the interdonation interval and the rate of decrement in serum ferritin values-longer the interdonation interval, less the decrement in serum ferritin levels. A significant corelation was also found between the sge groups and serum ferritin levels. The donors need to be educated about the iron deficiency and need for diet modifications. Iron supplementation can be given to donors donating blood on regular basis to avoid the loss of a safe donor pool because of iron deficiency which can be easily prevented by timely interventions.

Keywords: Voluntary blood donors, Hemoglobin, serum ferritin, subclinical iron deficiency

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INTRODUCTION

Blood donation is a life saving humanitarian act towards the sick. Blood donors are the foundation on which any transfusion service is able to function smoothly. Donor safety, patient safety and sufficient supply of safe blood and blood products are the three basics on which the transfusion services work upon to ensure the health and safety of both the donor and the recipient.

Initially, the concept of blood donations started in the beginning of the twentieth century but the anonymity of the donor was not kept.[1,2] As the advances occurred in the field of transfusion medicine,the ethical principles of donation came to be discussed at various forums. In 1952, the French government passed a law stating the blood donation process to be entirely an anonymous and voluntary process which was to be of no profit to either the donor(i.e.unpaid donations;no renumeration in cash or kind) nor to the operator,this being referred to as the not-for -profit blood donation system.[3] Since then, the Voluntary Non Renumerated blood donation(VNRD) became the standard for blood collection and paid donations were forbidden by international laws. Considering the various hazards associated with paid and remunerated donations,the World Health Organization (WHO), the Red Cross/Red Crescent organizations, the European Directorate for the Quality of Medicines & Health Care (EDQM), the European Blood Alliance (EBA), along with numerous Non Governmental Organizations (NGOs), national and international transfusion societies (such as the International society of blood transfusion [ISBT]) and blood donor associations [the International Federation of Blood Donor Organizations (FIODS)] pledged that all nations adhere to the VNRD model by 2020 (postponed to 2025 for certain regions such as Middle-East).[4,5]

WHO defines a regular voluntary non-remunerated blood donor as one who has donated at least three times, the last donation being within the previous year, and continues to donate regularly at least once per year.[4] Worldwide, blood centers are working upon recruiting regular

voluntary donors to meet up with the increased demands of blood and blood components. Nearly 70% of the blood donations comes from regular committed donors. Thus the well being, health and safety of the blood donors is of paramount importance to the medical profession.

A donor generally donates approximately 450/350 ml blood at the time of donation on basis of donor weight i.e. 350 ml for 45-55 kg and 450 ml for >55 kg weight. This results in a loss of approximately 200-250 mg of iron. This loss takes about 6 months to be replenished in absence of iron supplementation in both male and female donors.[6,7] According to the recommendations of NACO/NBTC and the Drugs & Cosmetics (Second Amendment) Rules,2020, a healthy individual with haemoglobin (Hb) levels ≥12.5g/dl can donate blood at the interval of 3 months (in case of male donor) and at the interval of 4 months (in case of female donor).[8] It has been seen that iron stores can get depleted if blood is donated more frequently.

Iron deficiency is the leading nutritional deficiency disorder in the world with anemia being its common manifestation.[9] Roughly,50% of the cases of anaemia are caused by iron deficiency(WHO 2007) with Iron deficiency anaemia (IDA) being rated amongst the five leading causes of years lived with disability in humans.[10] The prevalence in India is projected to be around 65–75 %.[11] Absolute Iron Deficiency(ID)is defined as the reduction of total body iron stores (mostly in macrophages and hepatocytes) which if progresses can lead to IDA.[12] Absolute ID may occur in scenarios of increased demand, decreased intake, decreased absorption or malabsorption, or chronic blood loss. Amongst the causes of chronic blood loss,frequent blood donation is found to be the one along with other causes such as physiologic(menstruation in females), gastrointestinal infections, genitourinary infections, systemic causes(inherited coagulopathies etc). Blood donation is the most common cause of iatrogenic iron deficiency among healthy adults.[12,13] Iron deficiency amongst the blood donors is commonly due to repeated donations rather than a general nutritional deficiency.

A large number of regular blood donors develop negative iron balance that may eventually progress to IDA leading to potential loss of considerable number of regular blood donors who actually represents the best source of safe blood.[14] Provision of safe and adequate blood is an integral part of every country's national health care policy. Consequently, the health and well-being of blood donors are of paramount importance in transfusion medicine. Blood donor retention saves blood services institutions time and resources. Efforts to retain donors and get adequate blood for transfusion purposes are hampered by donor deferrals for various reasons: low hemoglobin being the major reason worldwide. Deferrals have cost and health care implications to blood services and a negative effect on the donors to give blood in the future. For this reason, blood services have a responsibility to protect the donors from landing into iron deficiency anemia.

Measurement of Hb is an easy, inexpensive and indirect way to rule out iron deficiency anemia in donors. But a large proportion of donors are iron deficient despite having hemoglobin levels above the cut off value of 12.5 g/dl.[15] Using only hemoglobin levels as screening methodsis insufficient and it does not reflect on the status of iron stores of an individual and providesno indication of the development of iron depletion. Therefore, more specific tests like serum ferritin, serum iron, TIBC, Transferrin saturation are required to identify the iron status of the donors.[16] Several studies have supported the use of serum ferritin as marker of measurement of iron stores.[17] Serum ferritin is described as a laboratory test of choice for ID screening by the WHO. The stability of ferritin in whole blood stored at 4 deg Celsius for upto 5 days further adds to the feasibility of measurement of serum ferritin for monitoring iron stores in blood donors.[18]

The purpose of this study is to assess the iron status and development of iron deficiency anemia in blood donors by using a combination of hematological and iron status parameters along with the assessment of the influence of frequency of blood donation on iron stores of the donor.

AIMS AND OBJECTIVES

1. To study serum ferritin levels in blood donors.
2. To study the effect of interdonation intervals on the serum ferritin levels in blood donors.
3. To study the correlation between the age groups of donors and change in serum ferritin levels.

REVIEW OF LITERATURE

Blood Transfusion Service (BTS) is a vital component of any health care delivery system. The time factor is very crucial in the availability of safe blood and blood products in health facilities where transfusion is performed.[19] Policy makers at different levels, programme managers and providers of blood for transfusion need to know the requirement of blood isfor their population and also the facts related to the areas and situations of requirement so that blood resources are optimally supplied and used.[20]

Social and economic development, along with increased health care coverage, has created shifts in the clinical demand for blood in, both developed and developing countries. Today, many countries including India are facing challenges in maintaining an adequate supply of safe blood and blood products and ensuring their appropriate use. In developed countries,the requirements for blood and blood components are being seen due to advanced health systems, sophisticated medical and surgical procedures, trauma care and management of blood disorders, and an increase in ageing populations.[21] But the recent reports from developed countries are indicating a decline in collection and utilization of blood.

However, the demand for blood and blood products seem to be still high in developing and underdeveloped countries. In general, the demand for blood in developing countries is mainly seen in areas of obstetrics(to support acute haemorrhage during pregnancy-related complications),paediatrics(new-born care especially in pre-term infants, management of sepsis, haemorrhagic disease of the new-born),medicine(chronic anaemia, HIV related anaemia, micronutrient deficiencies etc).[21] According to WHO, blood donation by 1% of the population is generally taken as the minimum need to meet a nation's basic requirements for blood.[22] As per the above norm, India's demand for blood is around 13.1 million blood units (1% of 1.3 billion population). Several studies and reports indicated varying amount of annual blood collection in India. Considering the mixed health care

system in India (public, private and not-for-profit) with a huge proportion of unregulated private sector, changing epidemiological and demographic pattern, inequitable distribution of health services, and ever-increasing population size, the estimation or forecasting of blood requirements in India has been a challenge.

The requirement of blood for the country is estimated to be 8.5 million to 10 million units/year, whereas the available supply is only 7.4 million units/year.[23] As per many studies and reports, a huge mismatch between demand and supply is evident.[23-26]

In order to meet the population need for blood, 62.3 per 1,000 eligible population (6.23% of the eligible population) should donate once in a year. The need is converted into a demand, only when the person seeks health care services in a health facility. The conversion of the need to demand depends on geographic, socio-economic, and cultural factors which affect the access to health services. However, it is essential to reduce the gap between need and demand which may not be just the health system's responsibility but, requires a multisectoral approach. According to this study, 34.3 per 1,000 eligible population (3.43% of eligible population) must donate blood once in a year to address the estimated clinical demand. The proportion can be reduced in the event of promotion of repeat voluntary non-remunerated blood donation. Addressing the clinical demand is the most critical aspect, as it reflects the current demand that is experienced in the health facilities. Failure to meet the current clinical demand indicates that the blood transfusion services in the country experience shortages.

With the rising demand for blood components in modern day medicine, the challenge for an efficient blood collection includes adequate supply of safe blood from voluntary, healthy, non remunerated and low risk donors. As per the information provided by 164 countries to the WHO Global Database on Blood Safety it is indicated that, worldwide, more than 92 million blood donations are collected on an annual basis. Of these, an estimated 1.6 million units are discarded due to the presence of infectious markers for transfusion transmitted infections(TTIs) i.e HIV, hepatitis B, hepatitis C and syphilis. Apart from the above ,an

addition of at least 13 million prospective donors are deferred from donating blood due to anaemia, existing medical conditions or the risk of infections that could be transmitted through transfusion.[27]

The deferral of whole blood donors can be either temporary or permanent. Deferrals can be characterized as temporary short term (1–56 days), long term (57–365 days), and multiple years/permanent (more than 365 days). In a developing country, like India majority of the donor population, is deferred due to temporary but easily correctable cause—Anemia.[28] The causes of anemia could be nutritional deficiency, anemia due to blood loss, anemia of chronic disease, and so on. Nutritional anemia is a worldwide problem with the highest prevalence in developing countries like India. By far the most common cause of nutritional deficiency is iron deficiency. It can be either due to inadequate intake or poor bioavailability of dietary iron (only 5%–10% is absorbed) or due to excessive losses of iron from the body (malaria/hookworm infestation). Losses related to seropositivity for infectious markers are well established. Donor losses due to other reasons, however, have not been extensively quantified.[29]

Iron is a trace element indispensable for all cell life. As a constituent of heme it participates in the synthesis of hemoglobin, myoglobin, cytochromes and enzymes essential for respiration, mitochondrial function and energy production. It is also a component of iron-sulfur clusters which are essential for the prosthetic group of enzymes involved in cell proliferation, DNA repair, heme synthesis and many other functions, including the activity of Iron Regulatory Protein 1 (IRP1) which, when acquires the iron sulfur cluster, ceases to control iron acquisition and acquires the role of aconitase, the enzyme that converts citrate to isocitrate in the tricarboxylic acid.[30] This inter-conversion strengthens the iron role in both metabolism and energy production. Iron is conserved in mammals. Iron derived from erythrocyte breakdown is quickly recycled to the circulating iron carrier transferrin by macrophages. Iron from damaged muscles is locally recycled to regenerating fibers[31] and iron released from damaged red cells in the circulation, e.g. in acute intravascular hemolysis, is recycled

by a “on demand” transient population of liver macrophages.[32] Virtually all body iron is reutilized prevalently in erythropoiesis. About 1 mg iron is lost daily and is replaced by an equal amount absorbed from duodenal enterocytes. The capacity to absorb iron may increase, but remains lower than the capacity of erythropoiesis expansion; this is one reason why IDA may easily develop after blood loss (or even blood donation), if iron stores are limited.

Several proteins are involved in intracellular iron trafficking. SLC25A37 encodes mitoferrin 1, a mitochondrial iron transporter that mediates iron flux from cytosol to mitochondria in erythroblasts, in response to the high requirements of iron for heme and hemoglobin synthesis.[33] SLC25A28 encodes mitoferrin 2, which transfers iron to mitochondria for heme and iron sulfur cluster biogenesis in nonerythroid cells. Poly (rC) binding protein 1 (PCBP1), a ubiquitous cytosolic chaperone, delivers iron to the storage protein ferritin.[34] The Nuclear Receptor Coactivator 4 (NCOA4) is emerging as the cargo protein that addresses ferritin to autophagosomes for degradation[35] in a process called “ferritinophagy” and has been reported to be important for the recovery of stored iron in case of need.[36] A tight regulation of the available iron is operated at both cellular and systemic level to ensure perfect homeostasis of the metal, providing adequate organs supply and avoiding both ID and iron overload.

Iron deficiency(ID) is defined as the condition in which iron stores are reduced or even depleted in the absence of overt anemia, indicating that iron supply to erythropoiesis is still maintained. Relative ID /iron deficient erythropoiesis (IDE)refers to conditions in which iron is lacking in specific tissues/organs, but total body iron is not decreased, reflecting an altered iron distribution. It should be strengthened that anemia is the easiest recognizable sign of ID. In principle lack of iron may affect tissues/organs other than erythropoiesis, as skeletal muscles, heart, brain, but ID of tissues other than erythroid marrow remains unrecognizable by using traditional tests. It is often claimed that symptoms as fatigue, muscle weakness, reduced physical performance, cognitive impairment may be due to muscle or brain ID.

However, at present the correct interpretation of these symptoms as signs of ID is only retrospective, when they abate after iron therapy.[37] Depending upon the etiology, ID may progress to frank anemia in some, but not in all cases. When IDA is the result of progressive ID, it usually develops rather slowly and for this reason may be well tolerated. The diagnosis of IDA requires laboratory testing, since symptoms may be present, but are unspecific and often are overlooked.[38] ID is common in blood donors, especially females, and limits the donations frequency.

ID can be diagnosed by simple tests. Interpretation of serum ferritin levels may be problematic when levels are increased, but when in the low range ferritin deficiency equals ID. In absolute ID, especially in the presence of anemia (IDA) ferritin is usually <15 ng/ml in adults and <12 ng/ml in children.[39] Levels lower than 30 ng/ml are accepted as a sensitive and specific cut off which correlates with the absence of iron stores in the bone marrow and thus identifies absolute ID.[40]

The total body iron (TBI) has been estimated to be at 50mg/kg in males (approximately 4000mg) and 40 mg/kg in females(approximately 2500mg).[41] About 70-80% of the TBI is found in red blood cells bound to hemoglobin. Nearly 10% of TBI is engaged in physiological functions such as enzymes, cytochromes or in bound form to Transferrin. Remaining 20% exists as iron stores (i.e. Ferritin) to be used for erythropoiesis and other metabolic functions.

Ferritin molecule is an intracellular hollow protein shell composed of 24 subunits surrounding an iron core that may contain as many as 4000-4500 iron atoms. In the body, small amounts of ferritin are secreted into the plasma. The concentration of this plasma (or serum) ferritin is positively correlated with the size of the total body iron stores in the absence of inflammation. A low serum ferritin value reflects depleted iron stores, but not necessarily the severity of the depletion as it progresses. Normal ferritin concentrations vary by age and sex. Concentrations are high at birth, rise during the first two months of life, and then fall throughout later infancy.[42] At about one year of age, concentrations begin to rise again and continue to increase into

adulthood.[43] Beginning in adolescence, however, males have higher values than females; a trend that persists into late adulthood. Values among men peak between 30–39 years of age and then tend to remain constant until about 70 years of age. Among women, serum ferritin values remain relatively low until menopause and then rise.[43] Body ferritin levels, in contrast to haemoglobin, are not affected by residential elevation above sea level or smoking behaviour. However, ferritin is a positive acute phase response protein whereby concentrations increase during inflammation and thereby no longer reflect the size of the iron store. This makes the interpretation of normal or high serum ferritin values difficult in areas of widespread infection or inflammation.[39] In the absence of inflammation or liver disease, high serum ferritin concentrations indicate iron overload.

Addison et al(1972) developed the sensitive immunoradiometric technique which resulted in finding that ferritin is a constituent of normal human serum.

Thus it was determined that concentration of ferritin is found to be directly proportional to total iron stores in the body resulting in serum ferritin to become an important diagnostic tool in evaluation of iron status.[44]

Heinrich et al [45] summarised in their study that the physiological iron loss of a normal man totals 1.3 mg Fe/day or 489 mg Fe/year and after four blood donations of 410 ml (=213 mg of Fe) each per year ampunts to a loss of 1341 mg Fe/year. A daily uptake of 16.5 mg food iron (3000 Kcal/day) males absorb about 10 % or 1.7 mg Fe/day and 602 mg Fe/year, respectively. Four blood donations per year, therefore, produce an iron deficit of 739 mg Fe/year, so that the 800 mg of iron stores in a normal man are exhausted after one year. The increased physiological iron loss of menstruating women (1.8 mg Fe/day or 642 mg Fe/year) cannot be compensated by sufficient iron absorption from a typical, mixed diet, since only approx. 1.1 mg Fe/day (10%) are absorbed from daily food iron uptake of 11 mg (in 2000 Kcal/day). The already reduced reserves of 250 mg storage iron which are observed in about one half of the menstruating women are exhausted with the

induction of prelatent iron deficiency following one single blood donation of 410 ml. In the other half of menstruating women showing already completely depleted iron stores, 1-2 blood donations may lead to iron deficiency anaemia.

Finch et al in 1977[46] observed that one of the most common findings in long term blood donors is chronic iron deficiency. They studied the impact of iron depletion suggesting that male donors, while depleting their iron stores, were able to donate 2 to 3 units per year without appreciable incidence of iron deficiency, whereas women could only donate half that amount.

Skikne B et al [47] stated that the distribution of iron is amongst three compartments in the body i.e.

1. Storage compartment
2. Transport compartment
3. Functional compartment

Development of iron deficiency marks three consecutive stages of iron deficiency.

Stage 1: Iron depletion

Stage 2: Iron deficient erythropoiesis

Stage 3: Iron deficiency anemia

Deficiency of iron first affects the storage compartment resulting in iron depletion. Once the storage compartment is fully depleted, the transport compartment is affected resulting in insufficient supply of iron to bone marrow for erythropoiesis. Till this stage the condition is called subclinical iron deficiency and the hemoglobin levels remain normal. Iron deficiency anemia is manifested when the functional compartment is finally affected resulting in low hemoglobin levels.

Jacobs A. et al [48] stated ferritin to be a large spherical protein with 24 non covalently bound subunits having a molecular weight of approximately 450,000. These subunits form a shell surrounding a central core containing ferric hydroxyphosphate. Ferritin is the major storage protein of the body with each molecule of ferritin being capable of binding to about 4000-5000 atoms of iron.

Guyatt et al[49] concluded the serum ferritin radioimmunoassay to be an extremely powerful test for the diagnosis of iron-deficient anemia and, appropriately interpreted, can be applied to the complete range of patients. They interpreted the results of the study to alter clinical recommendations and practice in the diagnosis of iron-deficiency anemia. The first recommendation being that radioimmunoassay for the determination of serum ferritin concentration should be the only blood test ordered. Secondly, the traditional cutoff point dividing normal and abnormal, which in most laboratories is between 12 and 20μg/L, is not optimal. The likelihood of iron deficiency does not start to drop until values are higher than approximately 40 μg/l(for general populations) or 70μg/l (for those with inflammatory or liver disease). Third, the test needn't be abandoned in the management of patients who have inflammatory and liver disease, although a different approach towards the interpretation should be considered for such patients than for those without these conditions. Fourth, knowledge of the precise properties of serum ferritin can enhance the power of the laboratory diagnosis of iron deficiency.

Simon TL et al[50] observed that the loss of iron after each donation amounts to nearly a mean of 242 +/-17 mg for men and 217 +/-11 mg for women. After the donation there is a lag of 4 or 5 days before there is a change in plasma iron kinetics. Thereafter, plasma iron concentrations decrease and the clearance rate of iron from the plasma increases. Generally, iron stores are adequate between the first and second donations. After the second donation, however, an increase in iron absorption to sustain the increase of plasma iron turnover is required to maintain iron balance. Males with adequate iron stores, are generally able to maintain this balance and have less risk of landing into iron deficiency anemia. Even so, 8 percent of males with more than three donations per year will become iron deficient. These male donors will either continue to donate, often with Hb and/ or hematocrit levels that meet FDA and/or AABB requirements, but are diagnostic of anemia by clinical standards, or the donors become

deferred if their Hb and/or Hct levels drop below the 12.5 g or 38 percent requirement. Females especially postmenopausal females start with lower iron stores and thus more frequently become iron deficient after multiple donations. Between 17 and 28 percent of postmenopausal women will become iron deficient on a schedule of four to six donations per year. Iron deficiency is therefore a potential problem for all donors, but its major impact is on women who are having menstrual periods and undergoing childbirth. Unless they are taking iron supplementation regularly, even two donations per year are associated with a 28-percent incidence of iron deficiency. Thus moving ahead with a program of iron supplementation for blood donors would not only address the issue of inadequate blood donations to meet patient needs by providing a new source of regular donors, but it would also ensure that donors are not harmed by depleting their iron stores inappropriately. Iron deficiency is associated with symptoms and functional changes due to the critical role played by iron in human cell function. In addition, when the donors become anemic they are unable to donate, and the anemia may trigger a costly medical evaluation

A study done by Mittal R et al in 2006[51]stated that hemoglobin estimation alone is not adequate to detect subclinical iron deficiency states in regular blood donors. A significant decrease in serum ferritin levels(<15 ng/ml) was observed with increasing donation frequency (thrice per year) in both males(49%) and females(100%).

Mozaheb et al [52] when studying various stages of iron deficiency in donors, concluded that iron deficiency anemia was observed in 20% donors with three donations in one year. But measurement of only the hemoglobin level is not sufficient for selecting donors. In addition, testing of the ferritin level and iron supplementation are recommended in regular donors with more than one donation per year. The frequency of blood donation per year was found to be inversely correlated with hemoglobin, hematocrit, mean corpuscular hemoglobin concentration and serum ferritin. They found that

i) After blood donation the reduction of iron stores increases by frequent donations, and induces iron depletion and iron deficiency anemia.
ii) Just determining the serum hemoglobin is not a good measure for the state of iron stores, and it is not sufficient to ascertain the ability for donation.
iii) Measuring the serum ferritin level is the best test to evaluate the iron stores and can be used as a criterion to ascertain the donor's aptitude.

Measurement of serum ferritin is recommended when more than 1 donation is given per year. Furthermore, donors who donate more than once a year should receive iron supplement, except for those having multiple familymembers with cancer or a first-degree relative under the age of 60 years diagnosed with listed cancer.

Baart et al[53] observed that subclinical iron deficiency is prevalent amongst blood donors that have met the hemoglobin criteria for blood donation based on ZPP(Zinc protoporphyrin) levels and other iron variables such as ferritin, transferrin saturation, sTfr, hepcidin, MCV& MCH. It is important to detect subclinical iron deficiency not only to prevent the donors from landing into iron deficiency anemia but also to prevent degradation of various cellular processes since iron is an important constituent of many enzymes. The detection of subclinical iron deficiency can pave way for interventions such as dietary advice, iron fortification, prolonged interdonation intervals which can protect the donors from landing into IDA or other iron deficiency related disorders post blood donation. They also concluded that subclinical iron deficiency cannot be detected by using only hemoglobin measurement as screening tool. Thus it becomes imperative to search for alternate tests. Though the measurement of serum ferritin allows an optimized management of donors with subclinical iron deficiency along with the prevention of development of overt anemia. But it is accompanied with a drawback of increased values in cases of inflammation.

Gorakshkar et al[54] observed that the prevalence of iron deficiency increases with increased number of donations. Thus the blood

donations from such individuals is not justified. Several remedial measures were suggested such as screening by ferritin or free erythrocyte protoporphyrin(FEP) assay, increase in interdonation interval and oral iron therapy.

In 2012, Lee C.K. et al[55] conducted first study to assess predonation hemooglobin(PDH) and iron status in Chinese blood donors. An inverse relationship was observed between number of donations in previous 12 months and serum ferritin levels in 801 donors. The iron deficiency was observed in 35.1% of male and 65.3% of female donors who were deferred for low predonation hemoglobin levels and had donated at least twice in the previous 12 months. They concluded that it is crucial to monitor iron stores of vulnerable donors-whether by iron supplementation or by increasing donation intervals.

In studies done by Cable et al, 2012[56]; Rigas et al, (2014)[57] it was observed that endemicity of iron deficiency in blood donors affects about 25-35% of all blood donors. Increased prevalence of iron deficiency was observed in females, young donors, increased blood donation frequency and shorter time interval since last donation.

Cable et al [56] observed that the capillary hemoglobin overestimates the venous hemoglobin especially in donors with iron deficiency thus causing the worsening of already existing anemia.

In another study by Mast et al [58] conducted between June 2011 and April 2012,it was observed that amongst the 393 regular blood donors, worsening of iron deficiency anemia could be prevented if suitable interventions were done. In the absence of suitable interventions,the donors had worsening of iron deficiency in view of continued donation.

Ferraro et al [59] reviewed that most studies validating use of serum ferritin were performed before the introduction of the 2nd and 3rd WHO International Standards (ISs) to harmonize assay results. We revised the available literature to evaluate if consolidated clinical applications of ferritin and recommended cut-offs have been validated using ISs calibrated assays. All Medline retrieved reviews and individual studies performed since ISs availability were selected and analyzed according to predefined criteria. Concerning ferritin and iron deficiency (ID), only

one review, including studies published before 1988, met established criteria. Results showed that ferritin can effectively rule out ID anemia in patients with or without inflammatory disease at cut-offs of 70 and 40 μ g/L, respectively. From two studies using ISs calibrated assays that met inclusion criteria, no information emerged on which cut-off should be employed to obtain similar sensitivity. Regarding iron overload, even when the framework was restricted to hereditary hemochromatosis, no synthesis of scientific evidence, if any, about diagnostic accuracy of ferritin was available both before and after ISs introduction. Available evidence of the ferritin diagnostic effectiveness is limited to ID conditions.

A recent analysis as part of the REDS-II RISE study(2012)[60] found that anemia had a sensitivity of 10% to 44% and Hb had a low to moderate AUCROC (0.69-0.78 at enrollment) for detection of ID. The study showed that amongst the returning female first time/reactivated donors,20% and 515 had absent iron stores(AIS) and iron deficient erythropoiesis(IDE) at final visit respectively. The corresponding proportions for males stood at 8% and 20% respectively. The factors predicting IDE/AIS included higher frequency of blood donation in past 2 years, shorter interdonation interval, females and donors in younger age group. A converse relation was found in donors taking iron supplementation as it reduced the risk of iron depletion. The study concluded that the prevalence of iron depletion and hemoglobin deferral could be decreased by increasing the interdonation interval. As an alternate, replacement with iron supplements could allow for frequent donations without the risk of iron depletion.

Waldvogel-Abramowski et al[61] reviewed important physiologic pathwaysinvolved in iron metabolism of humans relevant to donor management in transfusion medicine. They concluded that ‘Ironomics’ may prove useful to better characterize patients with either iron deficiency or iron loading diseases. The term ‘ironomics’ has been used by investigators for analyzing the role of iron transporters among Yersinia pestis biotypes andYersinia pseudotuberculosis whereas the term ‘ironome’ has used by authors to describe iron metabolism and

trafficking within cells and organelles. Finally, ‘ironomics’ may be the ultimate goal for qualification and selection of individuals for blood donation according to their iron stores and of their capacity to maintain adequate iron metabolism despite supraphysiological iron depletion by blood donation.

Salvin et al in 2014[62] concluded that iron deficiency (ID) is an important consequence of blood donation. The epidemiology of this problem in the blood donor population was studied to enable appropriate targeting of potential solutions to donor ID. In a nationally representative cross-sectional study, the prevalence of ID and anemia was determined. The prevalence of ID exceeded most markedly in female whole blood donors, with important effects of donation frequency and age in this group. Female donors comprise 51 and 37% of the total Australian WB and apheresis (plasma and platelets) donation panels, respectively. Although multiple logistic regression models incorporating demographic characteristics and donation history could explain ID in high-risk donors, these models are unable to usefully predict ID in an individual donor. Similarly, although estimates of equivalentWB losses were associated with ferritin concentrations, they could not predict the risk of ID in an individual. Thus, beyond blood loss, other unmeasured dietary, environmental, or genetic (e.g., HFE,19 TMPRSS637 polymorphisms) factors influence donor iron stores. It was found that Hb is a poor predictor of ID, with only 59 donors failing to meet the Hb eligibility criteria for donation compared with 415 donors found to have ID. The performance of other RBC indices such as the proportion of hypochromic RBCs and reticulocyte Hb (measured using an automated hematologyanalyzer and thus not available in real time in a donation center) were somewhat better but were not diagnostic. Thus, measurement of capillary Hb, a strategy utilized worldwide, although useful to prevent phlebotomy of anemic donors, is not a reliable strategy for preventing venesection of iron-depleted individuals. As ID cannot be effectively predicted in the individual, direct measurement of iron stores in, for example, all premenopausal female donors who have made at least one donation in

the previous 12 months, would be desirable. However, the ability to measure donor iron stores efficiently and reliably at the point of care is limited. In Australia, the majority of collection centers are not equipped with diagnostic laboratories, nor other alternative suitable pointof-care platforms for the measurement of iron stores, precluding real-time ferritin measurement had been identified. Development of such tests could be of immense benefit for optimal targeting of ID control strategies

Rigas et al [57] conducted a large epidemiologic study on the influence of blood donation factors, physiologic factors, lifestyle and supplemental factors, and dietary factors on iron store levels and confirmed that premenopausal women were more prone to develop iron deficiency compared with postmenopausal women and men. In fact, among high frequent donors with more than nine donations in a 3-year period, a substantial proportion of donors and especially female donors were iron deficient.

Dara et al [63] conducted a study was to assess the efficacy of iron supplementation in maintaining the iron stores of voluntary blood donors. And concluded serum ferritin levels decreased significantly in relation to total annual donation frequency. In the study, the donors were given 3 weeks (21 days) of oral iron therapy (98.6 mg elemental iron/day) which improved Hb and serum ferritin levels initially, but the increase was not sustained. So, there is need to evaluate increased dosage and/or duration of iron supplementation, although increase in dose may decrease the compliance. In the absence of oral iron supplementation there is progressive reduction in iron stores. Policy for investigation and treatment of iron deficiency state and anaemia need to be incorporated as a part of voluntary blood donation program or as an alternative it can be included as a component of donor haemovigilance programme.

Mast et al [64] conducted a randomized, blinded, placebo controlled trial of education and iron supplementation for mitigation of iron deficiency in regular blood donors. In the study alternative approaches to maintain donor iron apart from offering dietary advice to donors with low

hemoglobin were explored. It was concluded that provision of 19 or 38 mg iron on a daily basis was effective for mitigationg iron deficiency in regular blood donors. In comparison, the donors without intervention had worsened iron deficiency with continued donations. The positive effects on iron status in subjects randomized to the iron status information group demonstrate that donors are interested and capable of modifying their behavior to prevent iron deficiency when provided accurate information about their iron status. Thus the key findings from the study are summarised as follows:

1) Once daily 19-mg iron pills are as effective as once daily 38-mg iron pills given for 60 days to mitigate iron deficiency in blood donors

2) Providing accurate information about iron status through measurement of ferritin and allowing the donor to choose whether to take iron supplements or delay donation is an effective method for mitigating iron deficiency in blood donors

3) Interventions to mitigate iron deficiency also improve donor Hb status in the context of frequent donation

4) Frequent donors who do not take actions to prevent iron deficiency become progressively more iron deficient with continued donation.

Therefore, directly providing iron pills to donors or providing iron status information in the form of a ferritin test result are operationally effective means that will mitigate iron deficiency in blood donors.

Rigas et al[57]reported in the 2 year follow up of Danish blood donor study and the iron supplementation programme,it was found that the goal-directed iron supplementation led to a general increase in blood donor Hb concentration and a reduced proportion of donors with low Hb concentration. Currently, the guided iron supplementation programme is still in place, and the other health regions in Denmark have implemented similar routine ferritin measurements. The repeat donors were sent mailed-out information and iron supplementation. However, with the increased effect of the programme the workload has levelled off, and currently, approximately 30% of women and 5% of men need iron supplementation and general information. In summary, the senior medical practitioner in charge of implementing the goal-

directed ferritin monitoring scheme concluded that even though the main goal was to keep donors within our limit for Hb and ferritin levels, a main benefit of the programme was to have a well-functioning programme for those who fell outside of Hb and ferritin levels. In general, either guided iron supplementation with screening for iron deficiency through ferritin measurements or adjusting the inter-donation intervals may alleviate the high proportion of iron-deficient donors. The experience from Copenhagen, the Capital Region of Denmark, is that guided iron supplementation is an effective strategy for avoiding iron depletion.

Tailor et al[65] in a study based in the western part of India observed that hemoglobin estimation alone may not be enough to evaluate donor safety prior to phlebotomy. In India, the iron stores in females are low especially in the reproductive age group. Hence, serum ferritin evaluation needs to be included in the testing of first time female donors for donor safety. Use of serum/plasma ferritin levels is considered to be the single, most powerful test for its diagnosis. Ferritin is independent of external contamination of blood samples, diurnal variation, and concurrent iron therapy. Even though serum/plasma ferritin is an acute phase reactant that can be elevated in various inflammatory conditions, as this study group comprised of healthy donor population, the probability of inflammation was negligible. For diagnosis of IDA, plasma ferritin threshold of 15 µg/ l was used. Considering the findings in this and other studies several measures to address iron depletion were considered. Measures such as:

1) Modifying the donor Hb requirements and measurement of Hb
2) Changing the inter donation interval
3) Testing for serum ferritin
4) Iron supplementation.

A national initiative to reduce the magnitude of iron deficiency in the population by education, behavioral changes in the area of food habits and iron supplementation is needed. Educating the donor about iron rich food through various educative materials to improve iron uptake through dietary modifications is necessary but the effect of this to

change the nature of food intake may be marginal. Supplementing iron tablets to regular donors can be thought. Oral iron is known to produce gastrointestinal side effects hence unlikely to be accepted by healthy donors even if it is given free from blood banks. However, in India a significant proportion of our population has haemoglobinopathy genes which on iron supplementation may cause iron overload. The ideal solution would be an education and supplementation of iron coupled with haemoglobinopathy control programme. A majority of Indians are vegetarian that results in poor absorption of iron from the food. Reduction of frequency of donation from 3-4 times a year to twice a year may bring down the proportion of donors who become iron deficient after a few years of regular transfusion. Finally, screening the donors by serum ferritin levels at the time of first donation and subsequently once every year becomes very rational for voluntary blood donation programme which can help to avoid iron deficiency in donor population. In fact, ferritin assay costs extra financial burden but can be valued on the other side by generation of national data on prevalence of iron deficiency, treatment of the iron deficient donor, referral of the donor for further medical investigation and treatment if donor remains persistently iron deficient in spite of compliant oral iron intake. This can also have a positive effect on donor safety & donor recruitment. Blood donation has a profound influence on iron stores and is a very important factor for iron deficiency in blood donors, particularly in multi-time donors and, especially in females despite of the fact that these donors are eligible to give blood according to the current guidelines (haemoglobin >12.5 g/dL). It may, therefore, be necessary to review the screening criteria used at the time of donation. It also seems logical to include serum ferritin measurement in the assessment of regular blood donors to secure adequate iron reserves in the donor population in order to maintain an appropriate donation potential.

Kiss et al in year 2018 [66] have concluded the need for assessing and implementing strategies affecting donor iron status which includes serum ferritin testing,increasing the inter donation interval as well as

advocating the use of low iron supplementation. Among blood donors with normal hemoglobin levels, low-dose iron supplementation, compared with no supplementation, reduced time to 80% recovery of the postdonation decrease in hemoglobin concentration in donors with low ferritin (≤26 ng/mL) or higher ferritin (>26 ng/mL).

Reddy et al [67] in a prospective study conducted from 2015-2017 demonstrated the presence of iron depletion in regular voluntary blood donors along with the presence of IDE in 11.2% of the donors. A ferritin screening after the first donation followed up at the tenth donation might help detect iron-deficient individuals. The mean ferritin values decreased in regular blood donors as early as after ten donations, implying that testing for serum ferritin should be done even at an earlier stage, preferably after five donations. This is to know the iron stores even before depletion. In this study, 83% of the female and 76% of the male donors with anemia (Hb<12.5g/dl) showed low ferritin values. This study also took into account the limitation that serum ferritin is an acute-phase reactant. Therefore, it is elevated in inflammatory conditions. Donors are screened prior to donation for any potential ill effects, but the inclusion of C-reactive protein would still rule out an increase in serum ferritin due to any other cause. This would be useful for a better interpretation of the results. Iron supplementation for all regular blood donors and female donors in particular will help prevent the shrinking donor pool due to iron deficiency.

The FIND'EM study by Sweegers et al [68] evaluated, by means of a nationwide stepped wedge cluster randomised trial, the effects of the ferritin-guided donation interval policy for whole blood donors on (1) Hb and ferritin levels, (2) low-Hb deferral, (3) donor return rates and (4) donor health (i.e. physical and mental wellbeing and iron deficiency- and donation-related symptoms). With respect to the duration of donation intervals, it was seen that iron-deficient donors may need tailored donation intervals but knowledge on optimal recovery times for iron-deficient donors is lacking. The deferral period is based on the study by Schotten et al.[69] in which recovery of ferritin levels in new and repeat male donors was suggested to require donation intervals of

180 days. If ferritin during the pre-donation screening is higher than 30 ng/mL, the donor is allowed to donate following regular donation schedules. If ferritin was found to be ≤ 30 ng/mL but ≥ 15 ng/mL, the donor was allowed to donate during the next visit to the blood collection centre, but ferritin will be measured again from the blood sample taken during their first whole blood donation. If ferritin during pre-donation screening is < 15 ng/mL, the donor is not allowed to donate for 12 months. After 12 months, the donor was allowed to donate but ferritin is measured again from the blood sample taken during their first whole blood donation. If the donor experienced complaints that could be associated with a low ferritin level, the new donor was advised to visit his/her general practitioner. For repeat whole blood donors, the intervention included ferritin measurements from blood samples taken at every fifth donation. When ferritin is > 30 ng/ mL, donors were eligible to donate at normal donation intervals. If ferritin is ≤ 30 ng/mL but ≥ 15 ng/mL, donors were deferred for 6 months. If ferritin was found to be< 15 ng/mL, donors were deferred from donating for 12 months. The use of a stepped wedge cluster randomised trial approach allows to implement and simultaneously evaluate the policy. As data collection takes place at multiple time points during the implementation period, all blood collection centres contribute data under both the control and intervention condition. Furthermore, this design makes it possible to investigate the effects as if it was a randomised controlled trial by correcting for time and clustering of donors and blood collection centres. This design also introduced some challenges, including prolonged trial duration compared to a standard randomised controlled trial, time-varying confounding, instructing all parties involved, making sure each cluster is committed to perform the assigned policy, and communicate with whole blood donors.

Gohel D et al [70] conducted a study and concluded that the current guidelines for blood donation i.e., predonation assessment of haemoglobin (Hb levels ≥ 12.5 g/dL)does not reflect true iron status of the blood donor. Thus more sensitive tests like Serum Iron and Serum

Ferritin in blood donors are important markers. Evaluation of iron stores was done as below:

Reduced at S. Ferritin values: Between 15-20 ng/mL

IDE at S. Ferritin values: <15 ng/ml but >12ng/ml

Iron Deficient Anemia at S. Ferritin values <12 ng/ml

Donors need to be educated about iron deficiency and recommended iron rich diet which can be easily available. Those who have donated more than twice should be put on regular iron supplementation to prevent the iron store depletion. In fact, a policy for investigation and management of iron deficiency anemia needs to be incorporated as a regular routine procedure in blood transfusion services. The results of the study provided an insight into the importance of monitoring the iron status of regular repeat donors, thereby, ensuring best of health care to donors and maintaining a regular pool of donors. Now, that the guidelines lay more stress and emphasis on regular repeated voluntary non-remunerated blood donations, the criteria for donor pre-donation testing needs to be revisited.

The blood banks are challenged with finding a balance between the increasing demand for blood transfusions and the difficulty of recruiting the new blood donors and retaining the regular blood donors. The potential for a regular donor to donate blood at regular intervals without developing iron deficiency anaemia varies with many factors such as difference in nutritional iron intake, the prevalence of iron deficiency in the defined population, the menstrual loss of iron in female, the frequency of donation, capacity for iron absorption and the use of supplemental iron.

Iron deficiency is a preventable side effect of blood donation. It restricts the resources of blood banks worldwide causing a permanent volunteer loss. For the optimum management of iron deficiency and prevention of anaemia, measurement of serum ferritin levels is beneficial. Assessing Serum Ferritin levels in the blood would be the most accurate way to assess iron store as it identifies the individual most at risk for iron depletion. Screening of Iron deficiency by estimating the serum ferritin has been recommended by WHO as the laboratory test of choice.

Because of the mixed levels of healthcare system in our country, there isa paucity of data regarding impact of regular voluntary blood donation on iron status of donors in India. This study has been undertaken to evaluate the iron stores by estimating serum ferritin levels among voluntary blood donors coming to blood center in tertiary care hospital in North India. This will be of help to identify the donors in the stage of depleted iron stores and take proactive measures before they develop iron deficiency anaemia.

MATERIALS AND METHODS

Place of study: Department of Immunohematology and Blood Transfusion, DMCH, Ludhiana

Duration of study: This prospective study was conducted over time period from January 2020-April 2021.

SOURCE OF DATA

200 voluntary blood donors coming for blood donationwere selected after screening them according to the donor selection criteria.

According to the age,the donors were distributed into four groups i.e.

Group A: Donors in the age group between 18-30 years.

Group B: Donors in the age group between 31-40 years.

Group C: Donors in the age group between 41-50 years.

Group D: Donors in the age group >50 years.

Based upon the interval between the last donation, the donors were divided into four groups-

Group I: Control group (n=50;males=35,females=15). This group included donors donating for the first time.

Group II: This group includes donors who have donated once in previous year (both males and females) and were now donating for second time (n=50). The inter donation interval in this group is 12 months.

Group III: This group includes donors(both males and females) who have donated twice in previous year and were now donating for third time (n=50). The inter donation interval for this group is 5-6 months.

Group IV: This group includes donors(both males and females)who had donated thrice in previous year and were now donating for fourth time(n=50). The inter donation interval for this group is 3-4 months.

METHOD OF COLLECTION OF DATA

All the blood donors were selected for blood donation as per the criteria laid down in Guidelines for Blood donor selection and blood donor referral, NACO (NBTC) 2017 Ministry of Health & Family Welfare, Government of India and modified according to the Drugs & Cosmetics (Second Amendment) Rules, 2020.

Predonation assessment of haemoglobin was done. Capillary hemoglobin was measured before each donation by photometry method with HemoQue Hb 301, Ängelholm, Sweden. The donors having hemoglobin≥ 12.5g/dl were selected for blood donation. A detailed proforma was completed for each donor after taking informed consent for participation in the study. Post phlebotomy, venous blood samples from study subjects were collected from diversion pouch at beginning of donation in 5 ml gel tubes for estimation of serum ferritin concentrations by Chemiluminescent Immunoassay(CLIA) technique using access ferritin assay(Beckman Coulter ACCESS Immunoassay Systems-Access Ferritin REF 33020). This assay is a two site immunoenzymatic "sandwich" assay used for quantitative determination of ferritin levels in human serum.

In this assay the reaction vessel contains goat anti-ferritin-alkaline phosphatase conjugate along with paramagnetic particles coated with goat anti-mouse: mouse anti ferritin complexes. The serum ferritin binds to immobilized anti ferritin on solid phase and the goat ferritin enzyme conjugate reacts with other antigenic sites on ferritin molecules. After incubation in the reaction vessel the materials bound to solid phase are held in magnetic field while the unbound particles are washed away. Addition of chemiluminescent substrate generates light which is measured by luminometer. The light production is directly proportional to ferritin concentration in sample

Evaluation of iron stores according to serum ferritin values was done as below:

Reduced Iron stores	: Between 15-20 ng/mL
Iron Deficient Erythropoiesis	: <15 ng/ml but >12ng/ml
Iron Deficient Anemia	:<12 ng/ml

INCLUSION CRITERIA

Donors fulfilling the eligibility criteria for whole blood donation as laid down in Guidelines for Blood donor selection and blood donor referral, NACO(NBTC)2017 Ministry of Health & Family Welfare,Government of India in accordance with Drugs & cosmetics Act 1940 and rules 1945

and as amended according to the Drugs & Cosmetics (Second Amendment) Rules, 2020 were included in study.

EXCLUSION CRITERIA

1. Donors receiving iron therapy
2. Donors having hemoglobin< 12.5 g/dl.

All the donors deferred according to other reasons than the ones listed above {as per those laid down in theDrugs & cosmetics Act 1940 and rules 1945 and as amended according to the Drugs& Cosmetics (Second Amendment) Rules, 2020} were excluded from the study.

STATISTICAL ANALYSIS:

Data were described in terms of range; mean ±standard deviation (± SD), frequencies (number of cases) and relative frequencies (percentages) as appropriate. Comparison of quantitative variables between the study groups was done using ANOVA and paired t-test if the distribution was normal and Kruskal Wallis test was performed in case the distribution was not normal. For comparing categorical data, Chi square ($\chi 2$) test was performed. For comparison of continuous variables with categorical, paired and unpaired t test was used. A probability value (p value)less than 0.05 was considered statistically significant. All statistical calculations were done using (Statistical Package for the Social Science) SPSS 21version (SPSS Inc., Chicago, IL, USA) statistical program for Microsoft Windows.

OBSERVATIONS AND RESULTS

A total of 200 donors were enrolled in the study. A demographic analysis of these donors showed 90%(180) male and 10 % (20) female donors. The male to female ratio was found to be 9:1.

The age wise distribution among the male blood donors was found to be 86.1% (n=62) in group A; 92.9%(n=79) in Group B;91.4% (n=32) in Group C and 87.5 %(n=7) in Group D. The mean age for males was found to be 34.1 ± 8.1 years. Amongst the female donors, the age wise distribution was found to be 13.9%(n=10) in Group A, 7.1%(n=6) in Group B, 8.6%(n=3) in Group C and 12.5%(n=1) in Group D. The mean age was found to be 32.8 ± 9.8 years for females. (Table 1)

Table 1. Age & Gender-Wise distribution of blood donors

Age (years)	**No. of Male Blood Donors n (%)**	**No. of Female Blood Donors n (%)**
Group A (18-30)	62 (86.1)	10 (13.9)
Group B (31-40)	79 (92.9)	6 (7.1)
Group C (41-50)	32 (91.4)	3 (8.6)
Group D (>50)	7 (87.5)	1 (12.5)
Mean age ± SD	34.1 ± 8.1	32.8 ± 9.8

Amongst all donors in the four classes of age groups, Kruskal Wallis test was performed for correlation between age groups and hemoglobin which yielded an insignificant difference amongst the two parameters(p=0.36) [Table 2]. The analysis done for correlation between age groups and serum ferritin levels did yield a significant result. (p=0.047).[Table 3]

Table 2. Comparison of Mean hemoglobin levels across age groups

Group	**Mean Hb (Sd)**	**P value**
A	13.74 (1.04)	0.36
B	13.68 (0.97)	
C	13.49 (1.10)	
D	13.55 (1.43)	

Table 3. Comparison of mean Serum ferritin levels across age groups

Group	**Mean S ferritin(Sd)**	P value
A	107.58(52.5)	0.047
B	86.09(58.41)	
C	80.74(61.47)	
D	76.11(48.58)	

When the values of serum ferritin were further analysed according to the different age groups,it was found that 2.5% of the donors of Group A and 3.5% donors each of Group B & C had serum ferritin levels < 12 ng/ml indicative of IDA .1% of the donors in group B(31-40 years) had IDE while 33.5 % donors of group A, 38% of Group B and 14 % donors of Group C had reduced iron stores.(Table 4) The age wise correlation done with different age groups and serum ferritin levels showed significantcorrelation(p=0.047).(Table 5)

Table 4 Distribution of Serum Ferritin levels according to age group

Group	**S.ferritin <12 ng/ml**	**S.ferritin 12-15 ng/ml**	**S.ferritin>15ng/ml**
A (18-30)	5	0	67
B (31-40)	7	2	76
C (41-50)	7	0	28
D (51-65)	0	0	0

Table 5 Showing correlation between age & Serum ferritin levels

Group	**S. ferritin N**	**Age**
S. ferritin N	1.0000	
Age	0.047	1.0000

Depending upon the interdonation intervals, the donors were divided into 4 groups. The mean hemoglobin was found to be 13.67±1.05 g/dl (Group I),13.77±0.99 g/dl (Group II),13.91 ±0.96 g/dl(Group III) and 13.32 ± 1.09 g/dl (Group IV) respectively. A p value of 0.029 was obtained after the comparison. In the individual group comparisons a significant p value was obtained for Group II vs IV (0.026) and Group III vs Group IV (0.004) (Table 6)[Figure 1]

Table 6 Comparison of Hemoglobin across various groups according to interdonation interval

	GROUP I		GROUP II		GROUP III		GROUP IV		F	p-value	I VS II	I VS III	I VS IV	II VS III	II VS IV	III VS IV
	Mean	SD	Mean	SD	Mean	SD	Mean	SD			p-value	p-value	p-value	p-value	p-value	p-value
Hb	13.67	1.05	13.77	0.99	13.91	0.96	13.32	1.09	3.06	0.029	0.599	0.239	0.089	0.514	0.026	0.004

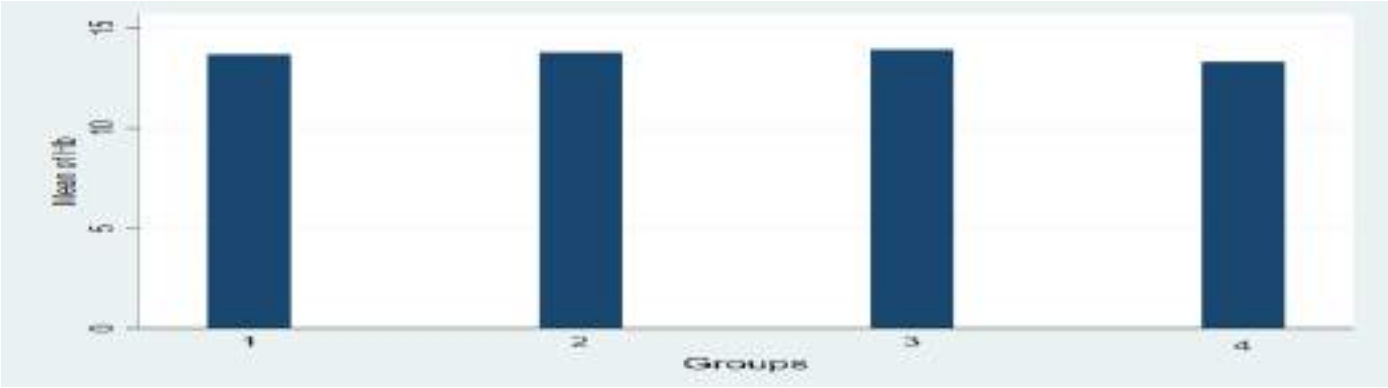

Figure 1. Mean value of hemoglobin across the groups

The donor samples were screened for serum ferritin levelsand the group wise results analysis was done. Group I,II,III & IV had mean serum ferritin levels at 83.82±49.37,101.04 ± 68.27,47.95 ± 35.40 and 27.30 ± 26.98 ng/ml respectively. [Figure 2] The comparison studies between the groups and their serum ferritin levels yielded a significant value i.e $p<0.05$ except for Group I vs Group II ($p=0.072$)indicating that a longer interdonation interval helps in preserving the iron stores of the body. The intergroup analysis between the other groups showed a significant decrement in levels of serum ferritin with the decrease in interdonation interval as shown in Table7.(Figure 3)

Table 7. Comparison of mean Serum ferritin levels across groups based upon interdonation interval

	GROUP I		GROUP II		GROUP III		GROUP IV		F	p-value	I VS II	I VS III	I VS IV	II VS III	II VS IV	III VS IV
	Mean	SD	Mean	SD	Mean	SD	Mean	SD			p-value	p-value	p-value	p-value	p-value	p-value
S.FERRITIN	83.82	49.37	101.04	68.27	47.95	35.40	27.30	26.98	24.71	0.000	0.072	0.000	0.000	0.000	0.000	0.031

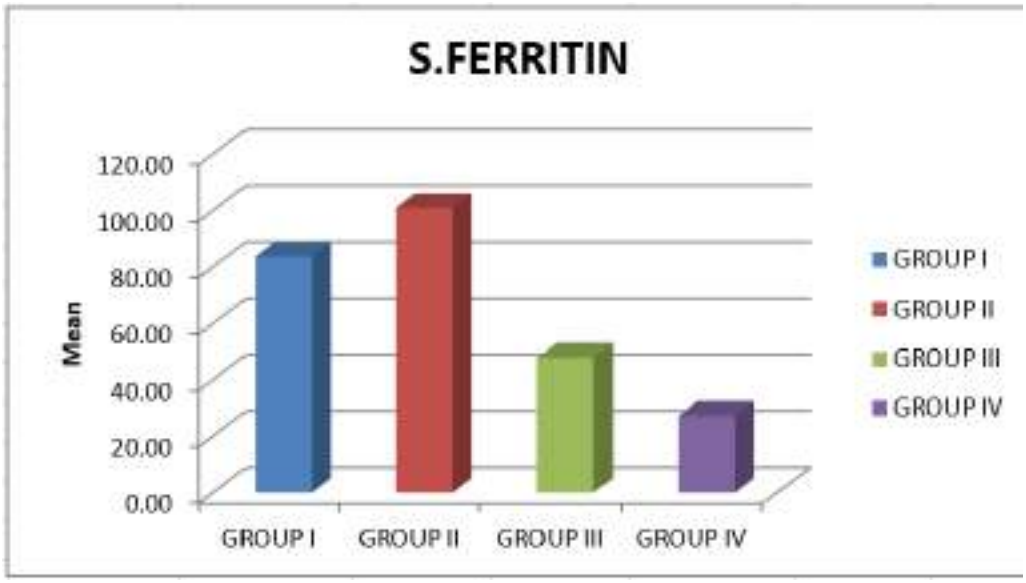

Figure 2. Mean value of Serum Ferritin across the groups based on interdonation intervals

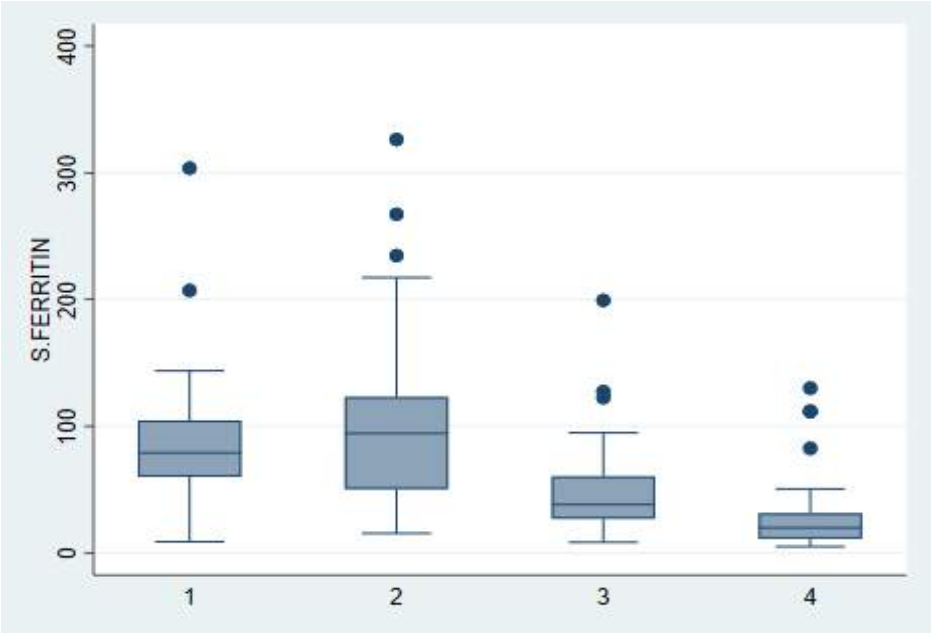

Figure 3 Box Plot Serum Ferritin among the groups

Further analysis of the serum ferritin levels amongst the groups showed that amongst the donors of Group I, 2 % (n=1) already had serum ferritin levels<12 ng/ml and 98%(n=49) had ferritin levels in normal range.

For Group II,98 %(n=49) donors had normal serum ferritin levels but 2 %(n=1) had reduced iron stores as depicted by serum ferritin levels between 15-20 ng/ml.

In Group III, it was observed that 4 %(n=2) of the donors each were reported for the serum ferritin values in the categorization of IDE & IDA,while 8 %(n=4) donors had reduced iron stores.

In Group IV, nearly 30 % donors(n=15) reported serum ferritin levels less than 12 ng/ml. This variation yielded a p value of 0.001 which is statistically significant.(Table 8)

Table 8. Table showing Classification according to Serum ferritin levels

		GROUP I		GROUP II		GROUP III		GROUP IV		Total	Chi-square value	p-value
		No	%age	No	%age	No	%age	No	%age			
FERRITIN	<12	1	2.0%	0	0.0%	2	4.0%	15	30.0%	18	60.105	0.0001
	<15 AND>12	0	0.0%	0	0.0%	2	4.0%	1	2.0%	3		
	15-20.0	0	0.0%	1	2.0%	4	8.0%	9	18.0%	14		
	Normal	49	98.0%	49	98.0%	42	84.0%	25	50.0%	165		
	Total	50	100.0%	50	100.0%	50	100.0%	50	100.0%	200		

In group I, which served as control group,there were males 70%(n=35)and females 30%(n=15). In Group II, there were 94% males(n=47) and 6% females (n=3). In Group III there were 96% males(n=48) and 4% females(n=2). Group IV showed 100% males (n=50).(Table 9)

Table 9: Gender-wise distribution of blood donor among groups

Groups	**No. of Male Donors**	**No. of Female Blood Donors**
Group I	35 (70%)	15 (30%)
Group II	47 (94%)	3 (6%)
Group III	48 (96%)	2 (4%)
Group IV	50 (100%)	0 (0%)

According to the analysis of serum ferritin based upon gender,it was found that amongst the male donors, 4 % donors in Group II,18% donors in Group III and 62 % donors in group IV had reduced serum ferritin levels. The variation of this parameter amongst the male repeat donors was found to be significant(p=0.0001).(Table 10)[Figure 4]

Table 10. Gender based distribution of serum ferritin

GENDER			GROUP				Total	Chi-square value	p-value
			I	II	III	IV			
F	REDUCED FERRITIN	No	15	3	2	0	20		
	Total		15	3	2	0	20		
M	REDUCED FERRITIN	Yes	0	2	9	31	42	62.567	0.0001
		No	35	45	39	19	138		
	Total		35	47	48	50	180		

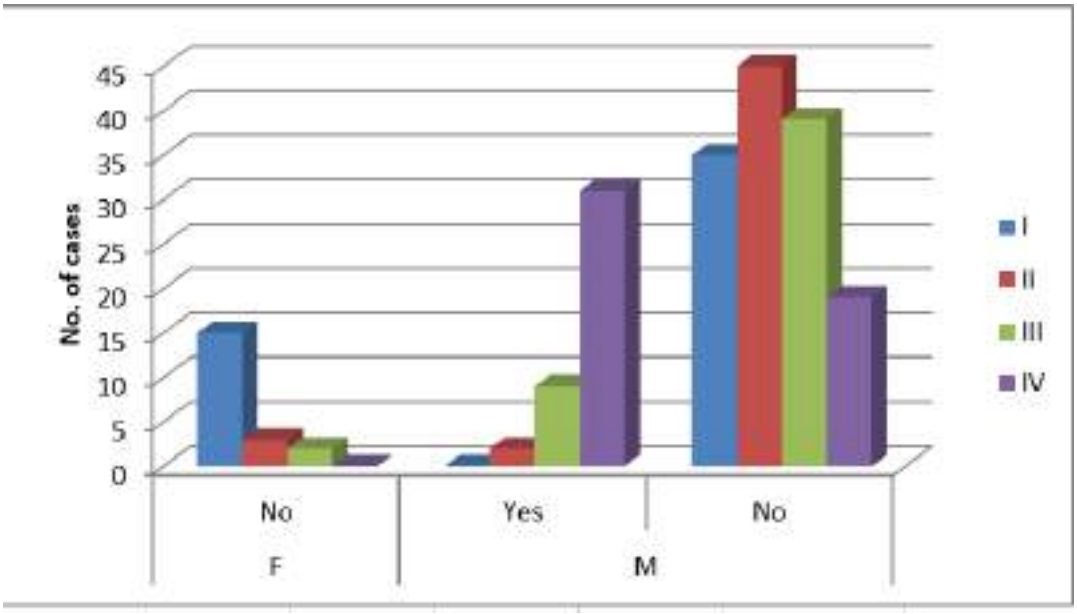

Figure 4: Graph showing gender based reduced ferritin levels across donor groups I-IV

Based upon gender, it was seen that in 0.5% of female donors and 9% of male donors across all groups showed the possibility of IDA with serum ferritin levels <12 ng/ml.(Table 11)

Table 11 Distribution of Serum Ferritin levels based upon gender

Gender	S.ferritin<12 ng/ml	S.ferritin 12-15 ng/ml	S.ferritin>15ng/ml	Total
Female	1	0	19	20
Male	18	2	160	180

For the male donors, a significant p value for hemoglobin levels(p=0.01) was noticed between Group I ,II,IIIvs Group IV and in Group II vs Group IV. The hemoglobin levels though above 12.5 g/dl showed a decrease in their mean values with increase in frequency of donations and decrease in interdonation interval (Table 12). On a similar pattern, a significant correlation was seen in the comparison between serum ferritin values of all groups (p <0.05) except for Group I vs Group II(p=0.119) (Table 13)[Figure 5]

Table 12 Comparison of hemoglobin in males across different groups as per last donation interval

Male	GROUP I		GROUP II		GROUP III		GROUP IV		F	p-value	I VS II	I VS III	I VS IV	II VS III	II VS IV	III VS IV
	Mean	SD	Mean	SD	Mean	SD	Mean	SD			p-value	p-value	p-value	p-value	p-value	p-value
Hb	13.81	0.98	13.80	1.01	13.97	0.94	13.32	1.09	3.826	0.011	0.972	0.472	0.028	0.415	0.019	0.002

Table 13 Comparison of Serum Ferritin in males across different groups as per last donation interval

Male	GROUP I		GROUP II		GROUP III		GROUP IV		F	p-value	I VS II	I VS III	I VS IV	II VS III	II VS IV	III VS IV
	Mean	SD	Mean	SD	Mean	SD	Mean	SD			p-value	p-value	p-value	p-value	p-value	p-value
S.FERRITIN	89.07	47.64	105.45	68.06	49.13	35.64	27.30	26.98	27.43	0.000	0.119	0.000	0.000	0.000	0.000	0.022

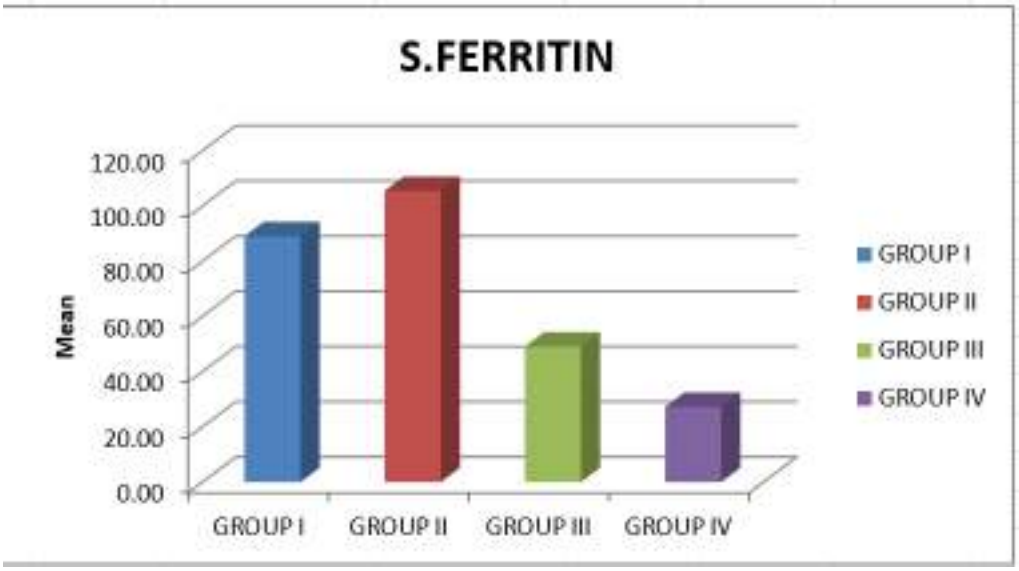

Figure 5: Graph showing gender based mean of serum ferritin values across Groups I-IV

In female donors coming for blood donation, a p value of 0.59 and 0.22 concluded respectively for hemoglobin and serum ferritin values indicated no significant difference amongst the four groups for female donors.(Table 14& Table 15)

Table 14 Comparison of hemoglobin in females across different groups as per last donation interval

Female	GROUP I		GROUP II		GROUP III		GROUP IV		F	p-value	I VS II	I VS III	II VS III
	Mean	SD	Mean	SD	Mean	SD	Mean	SD			p-value	p-value	p-value
Hb	13.34	1.18	13.40	0.78	12.50	0.00			0.536	0.595	0.933	0.327	0.385

Table 15 Comparison of Serum Ferritin in females across different groups as per last donation interval

Female	GROUP I		GROUP II		GROUP III		GROUP IV		F	p-value	I VS II	I VS III	II VS III
	Mean	SD	Mean	SD	Mean	SD	Mean	SD			p-value	p-value	p-value
S.FERRITIN	71.55	52.80	32.03	6.83	19.75	6.29			1.646	0.222	0.210	0.170	0.783

All screened donors coming to donate who were taken up as a part of the study were counselled to donate at regular intervals to maintain a pool of regular donors. These counselling sessions were of benefit to maintain a healthy donor pool especially in the times of pandemic. Amongst the blood donors coming back for repeated donations, it was found that out of the 15 female donors who came to donate blood for the first time, 4 of them came back as repeat donors. Out of the four 2 came to donate after a period of 10-12 months. When their serum ferritin and hemoglobin levels for the Group I vs Group II were analysed, no significant difference was found.(p=0.5). The remaining 2 donors out of the four returned after a period of 4 months. The analysis of serum ferritin values led to a very weak/negligible correlation in the decremental values(p=0.09).(Table 16) [Figure 6 & Figure 7]

Table 16: Comparison of hemoglobin and serum ferritin levels in females coming for repeat donations

Female	N	Minimum	Maximum	Mean	Std. Deviation	t	p-value	Mean difference	SD Difference
Gp I Hb	4	13	14	13.20	0.57				
Gp II Hb	2	13	13	12.95	0.35	1.000	0.500	0.65	0.92
Gp III Hb	2	13	13	12.50	0.00				
Gp I Ferritin	4	24	47	36.01	9.07				
Gp II Ferritin	2	35	37	35.95	1.06	1.250	0.430	5.50	6.22
Gp III Ferritin	2	15	24	19.75	6.29	6.396	0.099	10.81	2.39

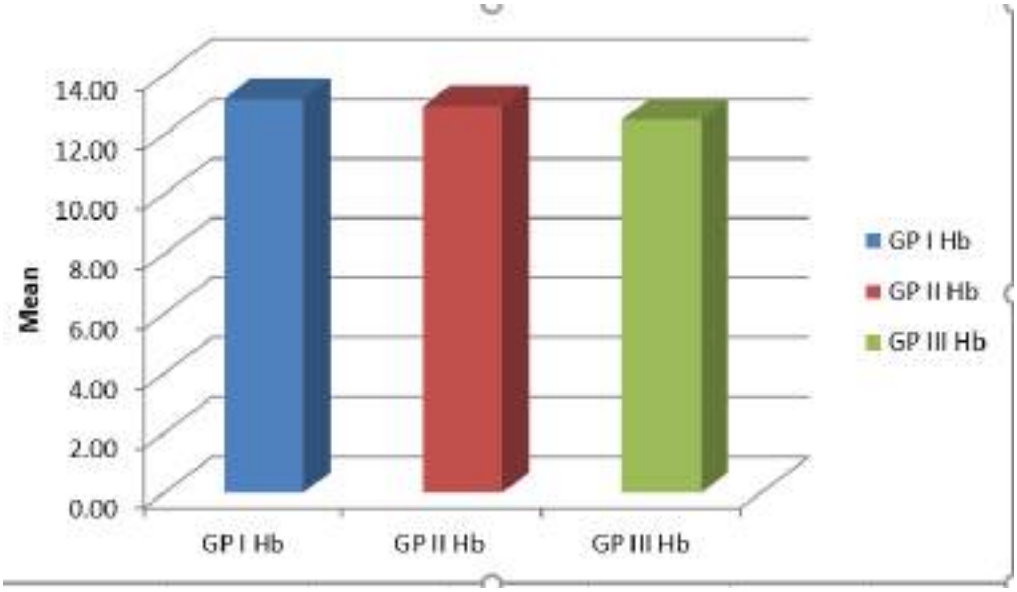

Figure 6: Comparison of mean hemoglobin levels for females coming for repeat donation

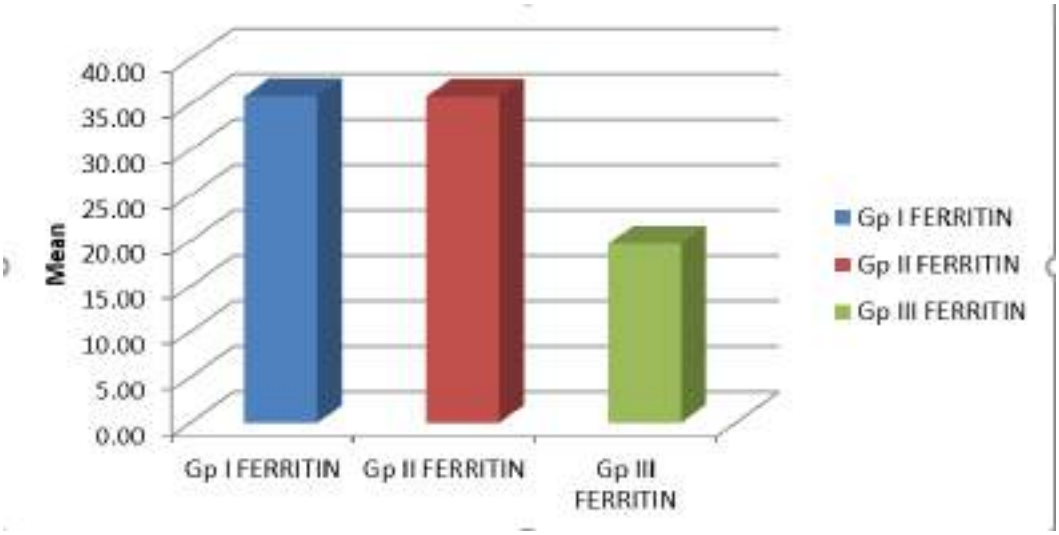

Figure 7: Comparison of mean ferritin levels for females coming for repeat donation

In a similar manner,15 out of 35 male donors came back for repeated voluntary donations. The mean hemoglobin and serum ferritin levels at time of first donation were 14.33±0.90 g/dl and 75.54± 29.36 ng/ml respectively. The hemoglobin levels and serum ferritin levels were analysed for these donors in a separate analysis. The mean hemoglobin and mean serum ferritin levels were found to be 13.77±0.82 g/dl and 51.31±20.16 ng/ml respectively. It was found that the 15 donors who came to donate after a period of 5-6 months had a significant decrease in hemoglobin levels(p=0.002)even though the

levels remained above normal i.e.>12.5 g/dl. The serum ferritin levels for these 15 donors though it remained in the normal range but showed a significant decrease when compared amongst the 2 groups.(p=0).

A repeat blood donation was seen for 10 out of the 15 donors. The mean hemoglobin and serum ferritin levels turned out to be 13.49±0.66 g/dl and 24.33±5.54 ng/ml. A significant correlation was seen for both hemoglobin and serum ferritin respectively.

When the hemoglobin and serum ferritin values were compared for the first and the subsequent donations made by these donors, a significant difference was seenamongst the serum ferritin levels amongst groups of people donating for first timeand the subsequent donations after 6 months and 3 months.(Table 17)[Figure 8 & Figure 9]

Table 17: Comparison of hemoglobin and serum ferritin levels in males coming for repeat donations

male	N	Minimum	Maximum	Mean	Std. Deviation	t	p-value	Mean difference	SD Difference
Gp I Hb	15	13.00	15.90	14.33	0.90				
Gp III Hb	15	12.50	15.00	13.77	0.82	3.824	0.002	0.55	0.56
Gp IV Hb	10	12.50	14.60	13.49	0.66	6.166	0.000	1.18	0.61
GP I Ferritin	15	32.50	135.90	75.54	29.36				
GP III Ferritin	15	16.68	88.93	51.31	20.16	6.714	0.000	24.22	13.97
GP IV Ferritin	10	16.70	36.03	24.33	5.54	5.435	0.000	53.57	31.17

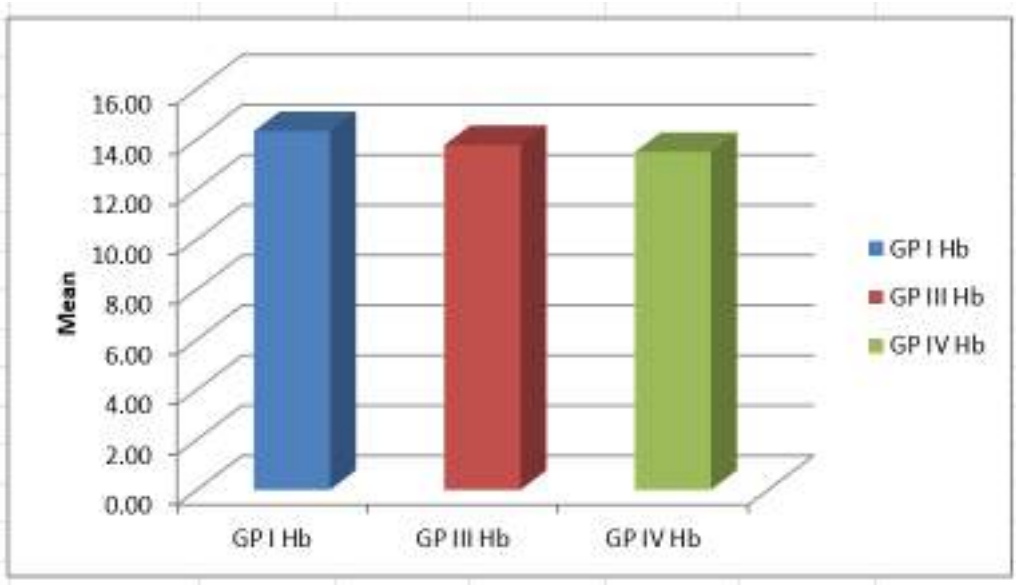

Figure 8: Comparison of mean hemoglobin levels for males coming for repeat donation

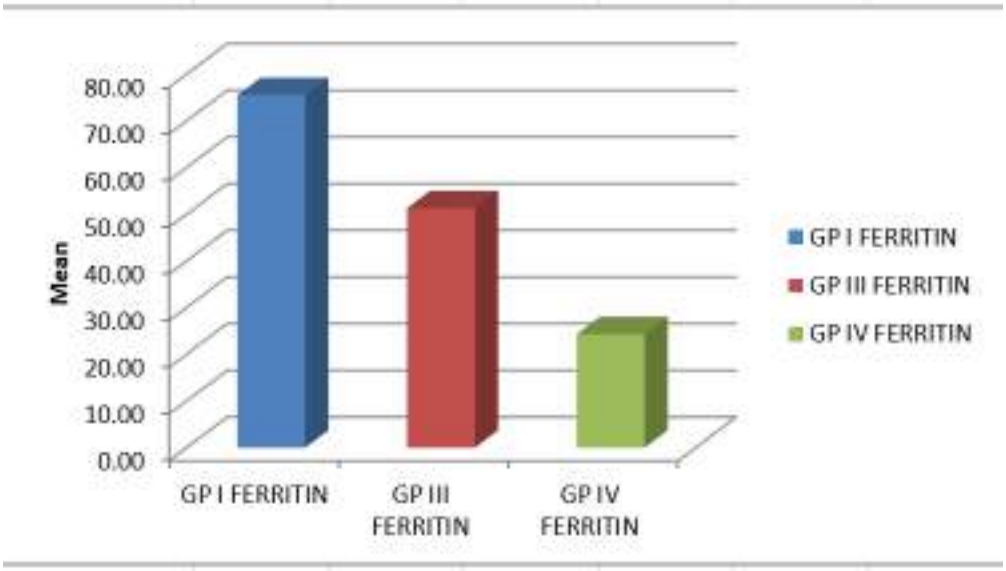

Figure 9: Comparison of mean ferritin levels for males coming for repeat donation

DISCUSSION

An adequate and safe supply of blood depends on the healthy individuals who can cope up with blood donations on regular basis. Although, repeated regular blood donation is considered a good donation and in order to fulfill the necessities, blood centers find it more effective to collect blood from existing donors than to recruit new donors but this strategy leads to iron depletion of these donors.

The prevalence of nonanemic iron deficiency is prevalent in blood donors especially in frequent blood donors. In a study by Zulfiqar et al[71], it was observed that 10% of the patients had normal Hb levels despite low serum ferritin (<10ng/mL). This shows that normal Hb levels do not exclude iron deficiency. Gohel et al[70] also concluded in their study that nearly 6.94 % of repeat blood donors had Serum ferritin levels <12 ng/ml. Our study also showed that 19 % of the blood donors had serum ferritin levls<12 ng/ml despite of normal predonation hemoglobin values.

Alvarez L. et al[72] in a retrospective analysis of 632 blood donors concluded that the serum ferritin levels for first time male donors (145.3 ± 9.9 µg/L)was found to be significantly higher than that found in regular male donors(35.3± 2.4 µg/L). In the population donating on regular basis 26 % of female and 5 % of males were found to be having IDA.72 % of the female and 85 % of male donors with anemia (i.e. Hb<125 g/L and 130 g/L respectively) showed ferritin levels lower than 30 µg/L. This provided enough clinical evidence for regular ferritin screening and early start of iron supplementation. In our study the analysis of serum ferritin based upon gender concluded that amongst the male donors, 4 % donors in Group II, 18 % donors in Group III and 62% donors in group IV had reduced serum ferritin levels. The variation of this parameter amongst the male repeat donors was found to be significant($p<0.05$).

Mozaheb et al [52] concluded that repeated blood donation have a significant effect on iron balance in all donors as conferred from a significant p value of <0.05 obtained after comparison between

hemoglobin and serum ferritin levels amongst the donor groups based upon the interdonation interval. Our study results also showed similar results for comparison both parameters amongst similar donor groups.(p value of <0.05).

Tailor et al[65] in an analysis done for donors in and around Surat,Gujarat concluded a mean ferritin concentration of 85.81 ng/ml in first time donors which was significantly higher than the mean value of 44.88 ng/ml among repeat blood donors. In female donors the mean ferritin concentration was 32.16ng/ml and that of male donors was 57.04 ng/ml. The results of this study showed that 28.5 % of blood donors had depleted iron stores (serum ferritin). Our study showed a mean ferritin concentration of 83.82 ± 49.37 ng/ml in first time donors compared to mean ferritin values of 47.95 ng/ml and 27.30 ng/ml for Group III and Group IV donors respectively. 17.5 % of the donors in our study had reduced ferritin levels.

Deepa et al[73] conducted a study with 88.2% males and 11.8% females. The distribution of donors on the basis of the frequency of donation per year were 50% first time, 23.9% once a year, 19.7% twice a year and 6.4% thrice a year donation. A statistically significant correlation was seen between frequency of donation and total number of life time donations and serum ferritin levels. Distribution on the basis of number of donations per year and serum ferritin resulted in a significant p value in the gender based analysis (males) of serum ferritin in donor groups I-IV.($p<0.05$). In our study there were 90% male and 10 % female donors. The variations amongst frequency of donations in a year and serum ferritin levels yielded a significant p value($p<0.05$)

Another study conducted by Reddy et al[67] enrolled around 374 voluntary blood donors and divided them into four groups based on the number of donations. This study concluded that for the total donors enrolled in the study, a population of around 9.8% were in Stage 1 of reduced iron stores; 11.2% were in Stage 2 (iron deficiency erythropoiesis) and 4.8% were in Stage 3(iron deficiency status with serum ferritin levels <15 µg/L and microcytic hypochromic picture on

the hemogram), respectively. For our study the donors were divided into 2 broad classification based on age and interdonation intervals. The categorization of serum ferritin levels led to the results that out of 200 donors enrolled in the study, 9.5 % had serum ferritin levels<12 ng/ml(indicative of latent stage of iron deficiency anemia),10 % were in the stage of IDE and around 15 % of the donors had serum ferritin levels indicative of reduced iron stores.

Gohel et al[70] found that the mean Hb level in all the donors was 14.178 g/dL. From the data obtained it is evident that amongst all four groups, maximum number of donors, who had reduced iron stores, iron depleted erythropoiesis and iron deficiency anemia belong to Group 2 (interdonation interval > 3-6 months) followed by donors of Group 1 (interdonation interval 3 months). There was a significant correlation between inter-donation and last donation interval with serum ferritin levels. Our study when compared amongst these parameters also gave similar findings.

As per age distribution of blood donors, it was concluded that amongst all four groups, maximum number of donors, who had reduced iron stores , iron depleted erythropoiesis and iron deficiency anemia belong to Group II (21-30 years) followed by donors of Group III (31-40 years)and IV (>40 years). In this study a significant correlation p<0.0001 was found for the comparison of hematological and serum ferritin levels as per the age distribution of the donors. Our study also showed a significant corelation (p=0.047) between the age distribution and the serum ferritin levels.

Ćatović-Baralija et al[74] concluded that a decrease of initial ferritin value in Group I relative to Group II was observed (without statistical significance). A significant decrease was found between repeated measurements for both hemoglobin and serum ferritin in both groups, indicating equal intensity of the decline in value regardless of a donor status. Measurement of ferritin before and after donation revealed statistically significant loss of ferritin in all examinees (p=0.011). The decline in haemoglobin after donation, although significant, did not fall below the reference value for donation in either women or men. Our

study also confirmed to the the result that a non significant p value was obtained for decrease in ferritin levels between Group I vs Group II. Measurement of serum ferritin in male repeat donors showed a significant decrease with subsequent donations.

Vhanda et al[75] analysed the effect of repeated blood donation on iron status of blood donors in Zimbabwe in which a total of 190 voluntary blood donors were enrolled of which over half were males (57%). Out of them 89% were repeat blood donors and 11% were donating blood for the first time. The median value of ferritin was 30 µg/L, which was significantly higher in males compared with females (40 vs 22 with p value< .001). Our study also showed that serum ferritin levels <12 ng/ml were seen in 0.5% of female donors and 9% of male donors across all groups.

Donors are a valuable asset to the blood centers. In order to recruit more donors, the donors coming for their first time donations were counselled to come back after a stipulated period for repeat donations. Out of the first time donors,38 % of the donors came back for a repeat donation out of which 21.05 % were females and 78 % were males. 10 % of these females came after 4-6 months and the analysis done revealed a weak decremental correlation in serum ferritin values. The increased frequency of donation was noticed amongst the male donors. An analysis of mean hemoglobin values and serum ferritin levels yielded a significant corelation($p<0.05$)

Table 18: Comparison between mean Hemoglobin and mean serum ferritin levels in first time and repeat donors between different studies

S. no	STUDY	NO.OF SUBJECTS		MEAN Hb IN DONORS(g/dl)		MEAN S. FERRITIN (ng/ml)		P VALUE
		FIRSTTIME	REPEAT	FIRST TIME	REPEAT	FIRST TIME	REPEAT	
1.	Alvarez et al (1999)	171	632	15.4	14.45	145.3±9.9	35.3±2.4	-
2.	Mozaheb et al (2011)	79	156	15.9	14.7	108	26	<0.000
3.	Tailor et al (2017)	111	282	13.58±1.37	13.15± 1.32	85.81±68.19	44.88 ± 56.51	<0.001
4.	Deepa DG. (2017)	157	157	14.4±0.91	14.2 ±1.22	47.52±37.6	20.5± 16.6	0.000
5.	Reddy k.(2019)	108	266	14.7±1.47	14.36±1.36	101.57±66.51	56.69±40.45	0.032
6.	Gohel et al (2021)	-	288	-	14.28±1.4	-	37.6±31.8	<0.05
7.	Present study	50	150	13.67±1.05	13.66±1.044	83.82±49.37	58.76±56.09	<0.05

This is in accordance to the observations made by various studies which documented that the frequency of donations made during a period of one year is more predictive of a decreased ferritin level rather than number of life time donations. An increase in donation frequency in one year is associated with a significant decrease in iron stores. There is a negligible effect on iron stores between the first and second donations. But with the increase in frequency of donation,there is a decrease in iron stores as a result of negative iron balance. In case of continued donations,the condition of negative iron balance can result in iron deficiency and anemia.[52]

Table 19: Comparison between mean Hemoglobin and mean serum ferritin levels for first time donors and their subsequent donations between different studies

S.no	Study	Mean Hb ±Sd (g/dl)		Mean S.ferritin±Sd (ng/ml)		p value
		First time	**Repeat**	**First time**	**Repeat**	
1.	Ćatović-Baralija et al	15.5±12.19	14.9±13.47	32.83(percentile)	23.36(percentile)	<0.05
2.	Present	14.33±0.90	13.77±0.82	75.54±29.36	51.31±20.16	<0.05

Current guidelines for blood donation require the screening only for hemoglobin levels i.e., Hb levels ≥ 12.5 g/dL. This is not a true indicator of the iron status of the blood donor. Therefore, more sensitive tests like S. Iron and S. Ferritin in blood donors need to be incorporated in the screening process especially for repeat voluntary donors. The results provide an insight into the importance of monitoring the iron status of regular repeat donors, thereby, ensuring best of health care to donors and maintaining a regular pool of donors. Now, that the guidelines lay more stress and emphasis on regular repeated voluntary non-remunerated blood donations, our criteria for donor pre-donation testing needs to be reevaluated.

SUMMARY AND CONCLUSION

In our study we found a significant correlation between the last donation interval and the serum ferritin measurement. An inverse relationship was found between the interdonation interval and the decrement in serum ferritin values-longer the interdonation interval, less the decrement in serum ferritin levels. The age wise correlation done with different age groups and serum ferritin levels showed significant correlation i.e. with increasing age, decrement in serum ferritin levels was seen in repeat donors despite the normal pre donation hemoglobin levels.

Thus the following conclusions can be made:

1. Blood donation has a profound influence on iron stores and is a very important factor for iron deficiency in blood donors, particularly in multi-time donors and, especially in female donors despite these donors being eligible to give blood according to the current guidelines.
2. The post donation decrease in haemoglobin , although significant, did not fall below the reference value for donation in either females or males and with such eligible predonation haemoglobin values voluntary donors would be eligible for the next donation, regardless of the expressed low ferritin value. But, regular donation would lead to depletion of ferritin stores and consequent iron deficiency anemia. This would cause the donor to be blocked for an extended period of time and thus a loss of healthy and safe donor pool.
3. Using predonation hemoglobin levels as the only screening method is insufficient and fails to identify the large number of iron-deficient donors as a high proportion of blood donors are iron-deficient or have reduced iron stores despite the eligible hemoglobin levels of blood donation
4. Guidelines lay more emphasis on safety of regular repeated voluntary non-remunerated blood donations, thus the criteria for donor pre-donation testing needs to be revisited

Some suggested strategies to maintain a healthy donor pool and to mitigate the ill effects of repeat blood donations on iron stores would be:

1. Screening the donors by serum ferritin levels at the time of first donation and subsequently once every year becomes very rational for voluntary blood donation programme to avoid iron deficiency in donor population.
2. Iron supplementation can be considered in all regular blood donors regardless of pre donation hemoglobin concentration.
3. Creating a strong IEC system to improve iron uptake can be beneficial. Education of the donor about dietary modifications, iron fortification can also show results.
4. There can be a substantial role of donor counselling in this part. BTS should consider counselling and iron supplementation for males as well, and not just for females, in order to keep a steady pool of voluntary donors at the base.

BTS in India rely on very fragmented mix of competing independent and hospital based blood banks of different levels of sophistication, serving different types of hospitals and patients. There is a need of uniform national guidelines which can be implemented through all levels of healthcare-be it in form of iron supplementation or reconsideration of prolongation of interdonation interval.

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KEY TO MASTER CHART

M	Male
F	Female
Hb	Hemoglobin
DOD	Date of Donation
Gp	Group
ID No.	Identification Number

S.NO	INITIALS	GROUP	AGE	DONOR ID No.	GENDER	DOD	Hb	S.FERRITIN
1	AK	I	42	41170	M	19.2.20	13.4	33.7
2	R	I	38	41338	F	22.2.20	12.8	8.8
3	JS	I	33	41809	M	28.2.20	14.2	71
4	KS	I	31	42352	M	5.3.20	14.4	60.8
5	NS	I	21	42409	M	6.3.20	15.9	80.5
6	BS	I	43	43032	M	15.3.20	14.8	32.5
7	KK	I	20	43126	F	16.3.20	13.5	36.3
8	PS	I	22	43178	M	17.3.20	14.2	66
9	GS	I	30	43181	M	17.3.20	14.2	65.2
10	GS	I	34	43182	M	17.3.20	14.3	113.9
11	PR	I	24	43329	M	20.3.20	14	100.3
12	AD	I	20	43334	M	20.3.20	15.2	22.6
13	AD	I	29	43391	M	21.3.20	14.5	78.1
14	HJ	I	35	44836	M	27.4.20	14.5	135.9
15	S	I	29	53734	F	7.10.20	12.8	25.18
16	M	I	29	53936	F	12.10.20	13	46.6
17	RS	I	45	53939	M	12.10.20	15	67.2
18	DS	I	43	54243	M	15.10.20	13	64.47
19	LL	I	33	54245	M	15.10.20	13.2	85.21
20	PD	I	35	54706	F	24.10.20	13.8	36.7
21	JT	I	30	54869	M	27.10.20	14.3	62.87
22	DS	I	22	54870	M	27.10.20	14.7	69.28
23	S	I	43	54887	F	27.10.20	12.5	24.42
24	BA	I	37	54888	F	27.10.20	12.5	78.72
25	RS	I	47	54978	F	28.10.20	12.5	87.2
26	RN	I	36	57097	M	28.11.20	13	36.91
27	SJ	I	31	60494	M	19.1.21	13.7	303.6
28	AK	I	38	60501	F	19.1.21	13.5	115.7
29	PK	I	57	60607	F	21.1.21	12.5	135
30	NS	I	35	64098	F	12.3.21	12.5	79.3
31	T	I	27	64099	M	12.3.21	12.9	70.5
32	S	I	25	64100	M	12.3.21	12.5	104.6
33	G	I	23	64101	F	12.3.21	13.7	60
34	VJ	I	25	64103	F	12.3.21	12.5	29.1
35	H	I	34	64105	M	12.3.21	12.9	66.7
36	D	I	38	65602	M	12.3.21	12.7	119.4
37	AS	I	30	65605	M	3.4.21	13.7	143.7
38	VJ	I	31	65672	M	3.4.21	14.6	105.3
39	S	I	35	65673	M	3.4.21	12.5	80.8
40	SU	I	36	65674	M	3.4.21	13	96
41	ASH	I	36	65675	M	3.4.21	12.5	90.53
42	SH	I	38	65676	M	3.4.21	12.6	85.4
43	G	I	36	65677	M	3.4.21	12.5	124.2
44	SK	I	26	65678	M	3.4.21	13.6	99.5
45	SUM	I	25	66123	M	13.4.21	12.7	54.13
46	P	I	41	66943	M	26.4.21	16	113.3
47	MP	I	24	66945	F	26.4.21	15.8	103.1
48	LP	I	28	66946	F	26.4.21	16.2	207.1
49	H	I	39	66947	M	26.4.21	14.2	84
50	S	I	42	66950	M	26.4.21	13.8	129.5
1	HS	II	37	41328	M	22.2.20	12.5	15.5
2	GR	II	41	41803	M	28.2.20	13.3	234.7
3	GS	II	26	42072	M	3.3.20	14.2	97.5
4	AS	II	25	42364	M	5.3.20	15.1	32.9

S.NO	INITIALS	GROUP	AGE	DONOR ID No.	GENDER	DOD	Hb	S.FERRITIN
5	AS	II	30	42411	M	6.3.20	15.1	30.7
6	PJ	II	21	51307	M	23.8.20	12.8	58.75
7	AN	II	27	53940	M	12.10.20	14.2	106
8	SK	II	39	54242	M	15.10.20	14.8	216.8
9	G	II	34	54246	M	15.10.20	13.8	115.9
10	IP	II	32	54707	M	24.10.20	12.6	20.94
11	RA	II	30	57042	M	28.11.20	12.5	96.8
12	AP	II	48	57088	M	28.11.20	14.6	139.4
13	MP	II	31	57102	M	28.11.20	13.8	183.7
14	NS	II	57	58302	M	28.11.20	16.5	174.5
15	SK	II	25	58233	M	28.11.20	14.4	165.3
16	ND	II	31	59687	M	7.1.21	15.1	119.9
17	DP	II	39	59693	M	7.1.21	14	49.43
18	VS	II	40	60504	M	19.1.21	13	190.4
19	AP	II	29	60605	M	21.1.21	12.8	102.6
20	HJ	II	45	60606	M	21.1.21	12.5	51.11
21	DP	II	29	60614	M	21.1.21	15.2	267.3
22	AK	II	35	60615	M	21.1.21	14.2	106.3
23	SS	II	44	60619	M	21.1.21	13.9	217.3
24	KS	II	32	60620	M	21.1.21	12.5	50.32
25	SJ	II	33	60653	M	21.1.21	14	85.18
26	JS	II	51	60744	M	23.1.21	12.5	103.9
27	PD	II	25	60745	M	23.1.21	13.9	156.5
28	ND	II	54	60757	M	23.1.21	14	123.1
29	RS	II	33	60761	M	23.1.21	16	326.2
30	MR	II	29	60870	M	25.1.21	14.2	109.9
31	DS	II	37	60911	M	26.1.21	14.7	57.25
32	HW	II	40	60921	M	26.1.21	13.5	92.29
33	GS	II	31	60925	M	26.1.21	12.8	113.6
34	MS	II	24	60941	M	26.1.21	15.1	51.43
35	PS	II	39	61932	M	7.2.21	15	137.5
36	PD	II	22	61937	F	7.2.21	12.5	24.2
37	PR	II	36	61948	M	7.2.21	13.2	97.1
38	ND	II	34	61954	M	7.2.21	13.1	43.8
39	SS	II	35	61969	M	7.2.21	13.8	78.1
40	KK	II	46	61981	M	7.2.21	13.6	60.8
41	KD	II	33	61984	M	7.2.21	12.5	64.3
42	SR	II	31	63490	M	1.3.21	14.5	96.4
43	MS	II	41	63491	M	1.3.21	13.3	67.6
44	GG	II	36	63497	M	1.3.21	12.7	57.8
45	MP	II	29	63498	F	1.3.21	13.8	36.7
46	HS	II	38	63762	M	6.3.21	13.2	43.6
47	KK	II	20	64102	F	9.3.21	13.9	35.2
48	KD	II	32	65604	M	3.4.21	13.6	63.67
49	ND	II	55	65606	M	3.4.21	12.5	41.9
50	RB	II	32	65681	M	3.4.21	13.4	40.03
1	BS	III	31	41289	M	21.2.20	15.4	14.3
2	SS	III	40	41290	M	21.2.20	13.8	49.9
3	KS	III	34	41454	M	23.2.20	14.3	8.4
4	BR	III	34	41461	M	23.2.20	14.3	52.4
5	PR	III	51	41479	M	23.2.20	14.6	81.5
6	RK	III	45	41486	M	23.2.20	13	8.5
7	HS	III	43	41787	M	27.3.20	15.1	27.7
8	RG	III	47	42085	M	3.3.20	12.5	12

S.NO	INITIALS	GROUP	AGE	DONOR ID No.	GENDER	DOD	Hb	S.FERRITIN
9	KS	III	28	42347	M	5.3.20	14.3	94.8
10	JS	III	31	42408	M	6.3.20	15.1	58.5
11	PK	III	40	42414	M	6.3.20	15.6	31
12	AS	III	31	43045	M	15.3.20	15.8	42.8
13	MS	III	32	43330	M	20.3.20	14.1	16.2
14	V.	III	39	43332	M	20.3.20	13.5	27.8
15	JK	III	21	43386	M	20.3.20	13	20.5
16	SV	III	25	43389	M	21.3.20	13.4	78.3
17	MK	III	35	44853	M	27.4.20	12.9	28.1
18	NS	III	21	51308	M	23.8.20	15	60.43
19	PS	III	22	51310	M	23.8.20	14.2	34.48
20	BS	III	43	54443	M	24.10.20	12.8	16.68
21	GV	III	27	54483	M	24.10.20	15.2	127.3
22	AD	III	29	54863	M	27.10.20	14.5	48.1
23	JS	III	33	54867	M	27.10.20	14.2	65.01
24	GS	III	30	54899	M	27.10.20	13.6	73.26
25	HJ	III	35	57083	M	29.11.20	13.8	88.93
26	AK	III	42	57356	M	2.12.20	12.5	25.8
27	PR	III	25	58311	M	18.12.20	13.9	70.7
28	GA	III	35	58231	M	18.12.20	14.2	36
29	KS	III	31	58232	M	18.12.20	14.2	60.8
30	JP	III	41	59689	M	7.1.21	14.6	93.98
31	HP	III	43	60492	M	19.1.21	13.6	20.89
32	BV	III	40	60500	M	19.1.21	12.9	122.5
33	SH	III	33	60505	M	19.1.21	16.2	52.08
34	AS	III	41	60621	M	21.1.21	13.5	199.3
35	HS	III	25	60746	M	23.1.21	12.9	27.03
36	RS	III	45	60764	M	23.1.21	15	59.85
37	NJ	III	25	60765	M	23.1.21	15.1	29.7
38	PS	III	26	60852	M	25.1.21	13.4	16.45
39	SU	III	25	60902	M	26.1.21	14.2	40.17
40	MJ	III	25	60903	M	26.1.21	14.5	40.17
41	LL	III	33	60915	M	26.1.21	13.2	48.78
42	SL	III	59	60936	M	26.1.21	13.3	37.56
43	DV	III	40	60938	M	26.1.21	13.6	32.77
44	BH	III	55	61931	M	7.2.21	12.5	28.6
45	S	III	43	61940	F	7.2.21	12.5	15.3
46	DS	III	43	61943	M	7.2.21	12.7	53.9
47	HP	III	43	61946	M	7.2.21	13.6	28.3
48	PD	III	35	61967	F	7.2.21	12.5	24.2
49	RN	III	36	63643	M	6.3.21	12.8	27
50	LP	III	24	65603	M	3.4.21	14	38.8
1	JS	IV	26	40811	M	16.2.20	12.5	30.6
2	GS	IV	41	40812	M	16.2.20	12.6	11.6
3	AS	IV	38	41167	M	19.2.20	13.8	8.6
4	HP	IV	37	41328	M	22.2.20	12.5	15.5
5	MS	IV	23	41481	M	23.2.20	13	111.9
6	MS	IV	38	41491	M	23.2.20	14.4	15.9
7	NK	IV	38	41720	M	26.2.20	14.4	26.4
8	RW	IV	43	42365	M	5.3.20	12.6	10.2
9	AT	IV	33	42353	M	5.3.20	16	82.5
10	HP	IV	35	42348	M	5.3.20	15.4	10.8
11	KS	IV	32	42412	M	6.3.20	12.6	6.1
12	PK	IV	25	43114	M	16.3.20	14.7	44.5

S.NO	INITIALS	GROUP	AGE	DONOR ID No.	GENDER	DOD	Hb	S.FERRITIN
13	YK	IV	35	43147	M	16.3.20	12.5	45.7
14	PK	IV	49	43148	M	16.3.20	13.2	7.3
15	HP	IV	40	43337	M	20.3.20	12.5	6.8
16	BS	IV	29	43386	M	21.3.20	12.5	16.1
17	GW	IV	28	44845	M	27.4.20	12.6	20.4
18	SS	IV	23	44846	M	27.4.20	12.7	19.4
19	JJ	IV	26	54425	M	24.10.20	12.6	10.5
20	HJ	IV	48	54874	M	27.10.20	12.8	130
21	CH	IV	32	54875	M	27.10.20	13.6	22.54
22	TJ	IV	41	54876	M	27.10.20	12.6	10.5
23	TV	IV	26	54900	M	27.10.20	16.4	111.4
24	AJ	IV	39	54982	M	28.10.20	12.8	45.07
25	PS	IV	22	57041	M	28.11.20	12.6	21.32
26	NS	IV	21	58238	M	17.12.20	14.6	36.03
27	GP	IV	22	58307	M	18.12.20	12.5	9.32
28	JV	IV	29	59688	M	7.1.21	12.5	33.18
29	SD	IV	29	59743	M	7.1.21	13.5	19.4
30	GS	IV	25	60490	M	19.1.21	12.5	26.83
31	GS	IV	33	60493	M	19.1.21	13.2	18.36
32	DP	IV	21	60639	M	21.1.21	12.6	18.97
33	JS	IV	33	60855	M	26.1.21	14.1	24.64
34	HJ	IV	20	60910	M	26.1.21	13.4	20.28
35	SD	IV	38	60918	M	26.1.21	12.5	11.16
36	BJ	IV	27	60920	M	26.1.21	12.6	10.09
37	HP	IV	35	60924	M	26.1.21	13	14.31
38	GP	IV	27	60937	M	26.1.21	12.5	9.3
39	SS	IV	30	61933	M	7.2.21	13.3	16.2
40	HS	IV	27	61951	M	7.2.21	12.5	5
41	BS	IV	43	63489	M	1.3.21	12.5	16.7
42	AD	IV	29	63492	M	1.3.21	13.8	23.4
43	AK	IV	42	63761	M	6.3.21	12.5	20.47
44	HJ	IV	35	64096	M	9.3.21	13	21.5
45	HK	IV	48	64097	M	9.3.21	16.8	11.4
46	BK	IV	45	65066	M	3.4.21	13	33.97
47	KS	IV	31	65601	M	5.4.21	13.6	31.29
48	GS	IV	30	66124	M	13.4.21	12.9	46.82
49	GA	IV	34	66133	M	13.4.21	13.6	23.98
50	RJ	IV	35	66948	M	27.4.21	14.9	20.65

Baba Farid University
of Health Sciences
Sadiq Road
Faridkot 151 203 (Pb). INDIA
Phone : 01639-256232,256236
Tele/Fax : 01639-256235
Email - Examinations@bfuhs.ac.in

No. BFUHS/2K21p-III/ 5025 Date 28/4/2021

The Principal,
DMCH, Ludhiana

Subject: **Approval of Plan of Thesis.**

This is to inform you that the Faculty of Medical Sciences in its meeting held on 22.01.2021 has approved the plan of thesis of **Dr. Arshpreet Kaur Batra**, a student of your college for MD Immuno **Haematology & Blood Transfusion** along with the names of Supervisor/Co-Supervisor as under:-

Topic Plan of Thesis
Study of serum ferritin levels in blood donors

Supervisor
Dr. Amarjit Kaur
Prof. & Head,
Dept. of I.H.B.T.,
DMCH, Ludhiana.

Co-Supervisor
Dr. Navjot Bajwa
Prof. & Head,
Dept. of Biochemistry,
DMCH, Ludhiana.

Dr. Rajesh Kumar
Prof.,
Dept. of I.H.B.T..

The student has to submit the three copies of thesis (along with PDF format) , to the Controller of Examinations, BFUHS, Faridkot, at least six months before the commencement of theory examination, if he/she is otherwise eligible to take the examination. He /She must directed to attach a photocopy of this letter with the forwarding letter while submitting thesis to the University along with the letter of approval of University for change of supervisor/Co-Supervisor, change of topic(if any).

Dr. S P Singh MS(Opth)
Controller of Examinations

Note :

1. ***If any discrepancy found regarding topic of thesis or Supervisor/Co-Supervisor, inform undersigned within one month after issue of this letter.***
2. *The student may submit the thesis as per the schedule already conveyed i.e. for May/June examination 30th Nov of the preceding the year of examination and for Nov/Dec examination 31st May of the year of examination.*

STUDY OF SERUM FERRITIN LEVELS IN BLOOD DONORS

THESIS PROPOSAL

FOR APPROVAL OF SUBJECT OF THESIS TO BE SUBMITTED IN

PARTIAL FULFILMENT OF THE REQUIREMENTS

FOR THE DEGREE OF

MD

In

IMMUNOHEMATOLOGY AND BLOOD TRANSFUSION

OF THE

BABA FARID UNIVERSITY OF HEALTH SCIENCES

FARIDKOT

SEPTEMBER, 2019

DR. ARSHPREET KAUR BATRA

DEPARTMENT OF IMMUNOHEMATOLOGY
AND BLOOD TRANSFUSION
DAYANAND MEDICAL COLLEGE AND HOSPITAL
LUDHIANA

ABSTRACT OF PLAN OF THESIS

Title	STUDY OF SERUM FERRITIN LEVELS IN BLOOD DONORS
For the degree of	MD (IMMUNOHEMATOLOGY AND BLOOD TRANSFUSION)
Name of the candidate	Dr. Arshpreet Kaur Batra
Supervisor	Dr. Amarjit Kaur, Professor & Head, Department of IHBT, DMCH, Ludhiana
Co-supervisor	Dr. Navjot Bajwa, Professor & Head, Department of Biochemistry, DMCH, Ludhiana Dr. Rajesh Kumar, Professor, Department of IHBT, DMCH, Ludhiana
Institution	Dayanand Medical College and Hospital, Ludhiana
University	Baba Farid University of Health Sciences, Faridkot

Introduction: Measurement of serum ferritin levels in regular voluntary donors helps to screen for subclinical iron deficiency. Early detection and timely interventions taken in case of subclinical iron deficiency prevents symptomatic iron depletion and also helps in preventing the donor loss to the blood bank because of iron deficiency anemia.

Aims & Objectives: To study serum ferritin levels in blood donors.

Material & Methods: A prospective analysis of hemoglobin and serum ferritin levels in 200 donors coming to Department of Immunohematology and Blood Transfusion for blood donation will be done. This includes the measurement of the same parameters in a control group of 50 healthy donors donating for the first time. The donors will include both sexes. A pattern between number of donations and serum ferritin levels and hemoglobin will be studied to assess the prevalence of subclinical iron deficiency.

Clinical Significance: Iron is needed for many physiological functions of the body. Repeat blood donations are associated with depletion of iron stores in body. Measurement of hemoglobin alone doesn't allow the exact assessment of iron stores in the body. To diagnose depletion of iron stores and hence to diagnose subclinical iron deficiency at an early stage, measurement of serum ferritin levels is required. This helps in preventing the repeat voluntary donors from landing into overt anemia and also helps the blood bank to maintain a healthy donor pool.

Keywords: Voluntary blood donors, Hemoglobin, serum ferritin ,subclinical iron deficiency.

(Signature)	(Signature)	(Signature)	(Signature)	(Signature)
Candidate	Co-supervisor	Co-supervisor	Supervisor	Principal

CERTIFICATE OF FACILITIES AVAILABLE

This is to certify that the facilities for work on the subject of thesis titled **"STUDY OF SERUM FERRITIN LEVELS IN BLOOD DONORS"** exist in the Department of IHBT, DMC&H, Ludhiana and will be provided to the candidate. We will see that the data being included in the thesis is genuine and is collected by the candidate herself under our supervision and guidance.

Supervisor

Dr. Amarjit Kaur
Professor & Head
Department of IHBT
DMCH, Ludhiana

Co-Supervisor

Dr. Navjot Bajwa
Professor & Head
Department of Biochemistry
DMCH, Ludhiana

Dr. Rajesh Kumar
Professor
Department of IHBT
DMCH, Ludhiana

Head of the Department

Dr. Amarjit Kaur
Professor & Head
Department of IHBT
DMCH, Ludhiana

Place: _____

Date: _____

APPROVAL PROFORMA
BY RESEARCH & ETHICAL COMMITTEE
DAYANAND MEDICAL COLLEGE & HOSPITAL, LUDHIANA

Name of candidate	Dr. Arshpreet Kaur Batra
Department	IHBT
Topic of Thesis	STUDY OF SERUM FERRITIN LEVELS IN BLOOD DONORS
Likely date of appearing in PG examination	May 2022
Date of enrollment	6th May 2019
Name of Head of Department	Dr. Amarjit Kaur
1) Supervisor **2) Co-Supervisor(s)**	Dr. Amarjit Kaur Dr. Navjot Bajwa Dr. Rajesh Kumar
Signature of Members of Research Committee with Stamp	**Signature of Members of Ethics Committee with Stamp**
Dr. Jaspal Singh (Professor & Head, Surgery)	Mr. A K Jindal (Member, Ethics Committee)
Dr. Dinesh Gupta (Professor & Head, Medicine)	Dr. Anu Sharma (Professor, Anatomy)
Dr. Sandeep Kaushal (Assistant Dean Academics)	Dr. Deepinder K Chhina (Professor & Head, Microbiology)
Dr. Rajoo Singh Chhina (Dean Academics)	Dr. Neena Sood (Professor & Head, Pathology)
Dr. Gurpreet S Wander (Vice Principal)	Dr. Gurpreet S Wander (Vice Principal)
	Dr. Gagandeep Singh (Secretary, Ethics Committee Professor & Head, Neurology)

Approved: Yes / ~~No~~

Dr. Sandeep Puri
Chairperson
Research Committee

Chairperson
Research Committee

Approved: Yes / ~~No~~

Prof. Arvind Malhotra
Chairperson
Ethics Committee

Chairperson
Ethics Committee

BABA FARID UNIVERSITY OF HEALTH SCIENCES, FARIDKOT

PROFORMA FOR REGISTRATION OF SUBJECT FOR DISSERTATION

1	NAME OF THE CANDIDATE AND ADDRESS	Dr Arshpreet Kaur Batra House no 815/2,Housing Board Colony, Guru Teg Bahadur Nagar, Jalandhar
2	NAME OF THE INSTITUTE	Dayanand Medical College, Ludhiana
3	COURSE OF STUDY AND SUBJECT	M.D. in Immunohematology and Blood Transfusion
4	DATE OF ADMISSION OF COURSE	6th May 2019
5	TITLE OF THE TOPIC	Study of Serum Ferritin levels in blood donors

6. BRIEF RESUME OF THE INTENDED WORK

6.1 NEED FOR THE STUDY

Donor safety, patient safety and sufficient supply of safe blood and blood products are the three basics on which the blood banks work upon. Worldwide, blood banks are working upon recruiting regular voluntary donors to meet up with the increased demands of blood and blood components. Voluntary non-remunerated donors are the cornerstone of safe, sustainable blood supply.[1] According to the World Health Organisation, all nations should make sure of 100% Voluntary Non –remunerated blood donation by the year 2020(2025 for Middle East regions).[2] Nearly 70% of the blood donated comes from regular committed donors. Therefore, safety and health of the blood donor population is of utmost importance.

Blood donation and to a small extent plateletpheresis is associated with loss of iron. Iron is needed for the development of erythrocytes as well as other physiological functions. Each blood donation resulting in withdrawl of a unit of blood(350/450 ml) causes a loss of iron in range of 200-250 mg.[3] This amount of iron needs a time period of about 24 weeks to be replaced on a standard diet without any supplements. Therefore repeat blood donors are at an increased risk of developing iron deficiency anaemia, in particular non anaemic iron deficiency (NAID) or very mild iron deficiency anaemia in contrast to severe iron deficiency anaemia.

Iron deficiency is one of the major problems faced by regular blood donors. Iron depletion has been associated with various non hematological symptoms such as pica, restless leg syndrome, cognitive changes.[4]

Measurement of capillary hemoglobin by fingerprick method has been used as pre qualifying criteria for blood donors worldwide. This prevents blood donation by anemic individuals. It has been documented in studies that capillary hemoglobin estimation underestimates the venous hemoglobin when the value is less than 12.5 g/dl.[5,6] This may lead to worsening of anemia in already anemic donors thus compromising on the values of donor safety.

Ferritin is a large spherical protein capable of binding to 4000-5000 atoms of iron making it major iron storage protein in the body.[7] Ferritin is a constituent of normal human serum. The concentration of serum ferritin is directly

proportional to the iron stores in the body. Therefore, measurement of Serum Ferritin in blood donors is a sensitive indicator of assessing the iron depletion in body which cannot be assessed by measurement of only hemoglobin. There is a decrease in serum ferritin levels after 10 donations and with increase in frequency of donations. Another reason for the decrease in serum ferritin can be occult blood loss as a result of some diseased conditions. Serum ferritin levels are reduced to one tenth in case of iron deficiency anemia. Therefore, Serum ferritin screening can be a useful tool in prevention of stage of iron deficiency in regular donors. This can also help in reducing the donor deferral rates in blood bank, thus contributing to healthy donor pool.

6.2 REVIEW OF LITERATURE

The beginning of 20th century marked the beginning of blood donations which were initially not kept anonymous[8-10] A law passed in France in 1952 mandated the blood donations to be kept voluntary, anonymous and on no profit basis either to donor or to the operator. This way Voluntary, non-remunerated blood donation became the standard for collecting blood and blood components.[2]

With the rising demand for blood components in modern day medicine, the challenge for an efficient blood collection includes adequate supply of safe blood from voluntary, healthy, non remunerated and low risk donors.[11-14] Therefore, it is very important and crucial for the blood banks to maintain a pool of healthy donors. Each whole blood donation (450 ml) is associated with iron loss of about 213-236 mg per unit of blood donated.[15]

Iron deficiency is one of the main causes of nutritional deficiencies worldwide. Anemia, being a common manifestation of iron deficiency can be used as a tool for assessing the prevalence of the same. Iron deficiency is responsible for 50% cases of anemia representing about 40% of the total iron deficient population

The total body iron (TBI) has been estimated to be at 50mg/kg in males (approximately 4000mg) and 40 mg/kg in females (approximately 2500mg). About 70-80% of the TBI is found in red blood cells bound to hemoglobin. Nearly 10% of TBI is engaged in physiological functions such as enzymes, cytochromes or in bound form to Transferrin. Remaining 20% exists as iron stores (i.e.Ferritin)to be used for erythropoiesis and other metabolic functions. Skikne B et al(1984) [16] states that the distribution of iron is amongst three compartments in the body and development of iron deficiency marks three consecutive stages of iron deficiency

Deficiency of iron first affects the storage compartment resulting in iron depletion. Once the storage compartment is fully depleted, the transport compartment is affected resulting in insufficient supply of iron to bone marrow for erythropoiesis. Till this stage the condition is called subclinical iron deficiency and the hemoglobin levels remain normal. Iron deficiency anemia is

manifested when the functional compartment is finally affected resulting in low hemoglobin levels.

According to WHO, anemia has been defined as the condition in which number of red blood cells or their oxygen carrying capacity is insufficient to meet the physiological needs of the body. These needs vary according to the age, sex, altitude, smoking and pregnancy status. The working definition of anemia in an adult includes the hemoglobin levels to be below 12.3g/dl(females) and below 14g/dl(males).[17] Based on international recommendations [17,18] iron deficiency has been defined as serum ferritin levels of less than 15ng/ml for both sexes

A study done by Mittal R et al in 2006 [19] stated that hemoglobin estimation alone is not adequate to detect sub clinical iron deficiency states in regular blood donors. A significant decrease in serum ferritin levels was observed with increasing donation frequency (thrice per year) in both males(49%) and females(100%).

In studies done by Cable et al, 2012a; Rigas et al, 2014, it was observed that endemicity of iron deficiency in blood donors affects about 25-35% of all blood donors. Increased prevalence of iron deficiency was observed in females, young donors, increased blood donation frequency and shorter time interval since last donation.

Cable et al, 2012b observed that the capillary hemoglobin overestimates the venous hemoglobin especially in donors with iron deficiency thus causing the worsening of already existing anemia.

In 2012, Lee C.K. et al observed an inverse relationship between number of donations in previous 12 months and serum ferritin levels was observed in 801 donors. The iron deficiency was observed in 35.1% of male and 65.3% of female donors who were deferred for low predonation hemoglobin levels and had donated at least twice in the previous 12 months.

Various studies (Radtke et al, 2005; Baart et al, 2013; Kiss et al, 2013) observed that other laboratory investigations such as serum ferritin ,serum iron/total iron binding capacity (%transferrin saturation), zinc protoporphyrin (ZPP)can be used to assess the iron status of the blood donors.

Another recently discovered variable which can be used for detecting subclinical iron deficiency is Hepcidin.[15] In various trials, serum ferritin has

been proven to be investigation of choice for screening iron deficiency by WHO.[20] Though soluble transferrin receptor is better than serum ferritin level as a screening tool [21] but its higher cost acts as a prohibitive factor in its implementation. The only drawback in using serum ferritin levels as a tool for measuring iron stores is that there might be a disproportionate increase in ferritin levels in chronic inflammation[22], acute and chronic liver disease, chronic renal failure and in some neoplastic diseases.[23]

In another study by Mast et al conducted between June 2011 and April 2012, it was observed that amongst the 393 regular blood donors, worsening of iron deficiency anemia could be prevented if suitable interventions were done. In the absence of suitable interventions, the donors had worsening of iron deficiency in view of continued donation.

Kiss et al in year 2018 have concluded the need for assessing and implementing strategies affecting donor iron status which includes serum ferritin testing ,increasing the inter donation interval as well as advocating the use of low iron supplementation.

Iron deficiency is a preventable side effect of blood donation. It restricts the resources of blood banks worldwide causing a permanent volunteer loss. For the optimum management of iron deficiency and prevention of anaemia, measurement of serum ferritin levels is beneficial.[24]

6.3 AIMS AND OBJECTIVES OF THE STUDY

1. To study serum ferritin levels in blood donors.
2. To study the effect of frequency of blood donation in development of iron deficiency in blood donors.

7. MATERIALS AND METHODS

Place of study: Department of Immunohematology and Blood Transfusion, DMCH, Ludhiana. Duration of study: 6 months prospective study

7.1 Source of Data

200 voluntary blood donors coming for blood donation.

The donors would be divided into **four groups-**

Group I: Control group (n=50;males=35,females=15).

It includes donors donating for the first time.

Group II: This group includes donors who have donated **once** in previous year (both males and females) and are now donating for second time (n=50). The inter donation interval in this group is one year (12 months).

Group III: This group includes donors (both males and females) who have donated **twice** in previous year and are now donating for third time (n=50).The inter donation interval for this group is 5-6 months.

Group IV: This group includes donors(both males and females) who have donated thrice in previous year and are now donating for fourth time(n=50).The inter donation interval for this group is 3-4 months.

7.2 METHOD OF COLLECTION OF DATA

All the blood donors are to be selected for blood donation as per the criteria laid down in Guidelines for Blood donor selection and blood donor referral, NACO (NBTC) 2017 Ministry of Health & Family Welfare, Government of India. (Proforma attached)

Predonation assessment of hemoglobin would be done by Hemocue Hb 301 method. All donors in the control group to have hemoglobin levels above 12.5 g/dl.

Post phlebotomy, serum will be separated from the pilot tubes from each donor and Serum ferritin levels will be measured using ELISA/ECLIA techniques.

INCLUSION CRITERIA

Donors fulfilling the criteria as laid down in Guidelines for Blood donor selection and blood donor referral, NACO (NBTC) 2017 Ministry of Health & Family Welfare, Government of India will be included in study.

EXCLUSION CRITERIA

1. Donors receiving iron therapy
2. Donors having hemoglobin< 12.5 g/dl.

NUMBER OF CASES

200 blood donors divided into 4 groups depending upon the frequency of blood donations done in the previous year.

OUTCOME: Serum ferritin measurement is an important tool in detecting iron deficiency anemia in blood donors. This allows for a systematic and optimized management of iron deficiency which can include steps like increasing the interdonation interval and iron supplementation. This can prevent the donors from landing into overt anemia.

STATISTICAL ANALYSIS: Data will be described in terms of range; mean ±standard deviation (± SD), median, frequencies (number of cases) and relative frequencies (percentages) as appropriate. Comparison of quantitative variables between the study groups will be done using ANOVA. For comparing categorical data, Chi square ($\chi 2$) test will be performed and exact test will be used when the expected frequency is less than 5. A probability value (p value) less than 0.05 will be considered statistically significant. All statistical calculations will be done using SPSS (Statistical Package for the Social Science) SPSS 21 version statistical program for Microsoft Windows.

7.3 DOES THE STUDY REQUIRE ANY INVESTIGATIONS OR INTERVENTIONS TO BE CONDUCTED ON PATIENTS OR OTHER HUMANS OR ANIMALS? IF SO, PLEASE DESCRIBE BRIEFLY.

Yes.

Pre donation hemoglobin levels –checked by Haemocue (Hb 301) method.

Serum ferritin levels need to be measured using the Access Ferritin Assay. The sample is to be taken from the sample collected in pilot tubes (EDTA) from each donor post donation.

7.4 HAS ETHICAL CLEARANCE BEEN OBTAINED FROM YOUR INSTITUTION IN CASE OF 7.3?

Yes

8. LIST OF REFERENCES

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PROFORMA FOR DONORS

S.NO

NAME

REGD NUMBER

AGE

SEX(MALE/FEMALE)

ADDRESS

CONTACT NUMBER

WEIGHT

BLOOD PRESSURE

HEMOGLOBIN (gm/dl)

HAVE YOU DONATED EARLIER(YES/NO)

WHEN WAS THE LAST BLOOD DONATION DONE?

SERUM FERRITIN LEVEL(ng/ml)

Signature of Candidate

Signature of Supervisor

INFORMED PATIENT CONSENT

Date: _____

I.....D/o, W/o, S/o..... .....am willing to participate in the study **"STUDY OF SERUM FERRITIN LEVELS IN BLOOD DONORS"** The procedure with its complications has been explained to me in local vernacular language. I have fully understood that no special tests and extra expenditure in any form, merely for the purpose of thesis is being carried out. I am free to opt out the study any time without assigning any reason.

....

Signature of the Witness Signature of the patient

Name:

Signature of Candidate **Signature of Supervisor**

मरीज अनुमति फार्म

तिथि _____

मैं _____ पत्नी/पुत्र/पुत्री _____

निवासी _____

इस शोधकार्य **"STUDY OF SERUM FERRITIN LEVELS IN BLOOD DONORS"** में भाग लेने के लिए सहमत हूँ। मैं इस शोधकार्य में भाग लेने के लिए अपनी स्वेच्छा से तैयार हूँ। मैं पुष्टि करता/करती हूँ की यह स्वीकृति देने के लिए मुझे कोई आर्थिक प्रलोभन नहीं दिया गया और न ही दबाव डाला गया है। मुझे इस शोधकार्य का उद्देश्य स्पष्ट कर दिया गया है और मुझे यह विश्वास है की यह विज्ञान और मानवता के लाभ के लिए है। मुझे इस शोधकार्य में होने वाले जोखिम के बारे में समझा दिया गया है और मेरी समझ में यह जोखिम बहुत मामुली है।

मैं इस शोधकार्य में भाग लेने के सम्बन्ध में किसी भी समय अपनी अस्वीकृति व्यक्त कर सकता/सकती हूँ और मेरे अस्वीकृति व्यक्त करने का मेरे इलाज पर कोई प्रभाव नहीं पड़ेगा।

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गवाह के हस्ताक्षर रोगी के हस्ताक्षर/अंगूठे का निशान

नाम: नाम:

_____

अन्वेषक के हस्ताक्षर

_____

विद्यार्थी के हस्ताक्षर

ਮਰੀਜ਼ ਰਜ਼ਾਮੰਦੀ ਫਾਰਮ

ਮਿਤੀ_____

ਮੈ_____ਪਤਨੀ/ਪੁੱਤਰ/ਪੁੱਤਰੀ_____

ਵਾਸੀ _____

"STUDY OF SERUM FERRITIN LEVELS IN BLOOD DONORS" ਤੇ ਚਲ ਰਹੀ ਖੋਜ ਵਿਚ ਹਿੱਸਾ ਲੈਣ ਲਈ ਰਜ਼ਾਮੰਦ ਹਾਂ। ਇਸ ਖੋਜ ਕੰਮ ਵਿਚ ਹਿੱਸਾ ਲੈਣ ਲਈ ਮੈ ਆਪਣੀ ਮਰਜ਼ੀ ਨਾਲ ਤਿਆਰ ਹਾਂ। ਮੈ ਇਹ ਦਸਣਾ ਚਾਹੁੰਦਾ ਹਾਂ/ ਚਾਹੁੰਦੀ ਹਾਂ ਕੇ ਇਹ ਰਜ਼ਾਮੰਦੀ ਦੇਣ ਵਾਸਤੇ ਮੈਨੂ ਕਿਸੇ ਵੀ ਤਰਾਂ ਦਾ ਲੋਭ-ਲਾਲਚ ਨਹੀ ਦਿੱਤਾ ਗਿਆ ਅਤੇ ਨਾ ਹੀ ਕੋਈ ਦਬਾ ਪਾਇਆ ਗਿਆ ਹੈ। ਮੈਨੂ ਇਸ ਖੋਜ ਦਾ ਮਨੋਰਥ ਸਮਝਾ ਦਿੱਤਾ ਗਿਆ ਹੈ ਅਤੇ ਮੈਨੂ ਇਹ ਭਰੋਸਾ ਦਵਾਇਆ ਗਿਆ ਹੈ ਕੇ ਇਹ ਖੋਜ ਵਿਗਿਆਨ ਅਤੇ ਮਾਨਵਤਾ ਦੀ ਭਲਾਈ ਲਈ ਹੈ। ਮੈਨੂ ਇਸ ਖੋਜ ਵਿਚ ਹੋਣ ਵਾਲੇ ਖਤਰੇ ਬਾਰੇ ਸਮਝਾ ਦਿੱਤਾ ਗਿਆ ਹੈ ਅਤੇ ਮੈਨੂ ਇਹ ਯਕੀਨ ਹੈ ਕੇ ਇਹ ਖਤਰਾ ਬਹੁਤ ਮਾਮੂਲੀ ਹੈ। ਮੈਨੂ ਇਸ ਖੋਜ ਕੰਮ ਵਿਚ ਹਿੱਸਾ ਲੈਣ ਦਾ ਢੰਗ – ਤਰੀਕਾ ਸਮਝਾ ਦਿੱਤਾ ਗਿਆ ਹੈ।

ਮੈ ਇਸ ਖੋਜ ਕੰਮ ਵਿਚ ਹਿੱਸਾ ਲੈਣ ਬਾਰੇ ਆਪਣੀ ਰਜ਼ਾਮੰਦੀ ਕਿਸੇ ਵੀ ਸਮੇ ਵਾਪਸ ਲੈ ਸਕਦਾ ਹਾਂ/ ਸਕਦੀ ਹਾਂ ਅਤੇ ਮੇਰੀ ਰਜ਼ਾਮੰਦੀ ਵਾਪਸ ਲੈਣ ਦਾ ਮੇਰੇ ਇਲਾਜ ਤੇ ਕੋਈ ਅਸਰ ਨਹੀ ਹੋਵੇਗਾ।

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ਗਵਾਹ ਦੇ ਹਸਤਾਖਰ ਮਰੀਜ਼ ਦੇ ਹਸਤਾਖਰ

ਮਿਤੀ_____ ਮਿਤੀ_____

ਪਤਾ _____ ਪਤਾ _____

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ਨਿਰੀਖਕ ਦੇ ਹਸਤਾਖਰ

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ਸਿਖਿਆਰਥੀ ਦੇ ਹਸਤਾਖਰ